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BIOLOGICAL LECTURES

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PREFACE.

In offering the second volume of these lectures,[1] it may be well to remind the reader who may not be acquainted with the Laboratory, that only *one* side of our work is here represented. Such a series of lectures on special problems in biology is offered every summer; but the main body of our lectures is of quite a different type, having direct reference to the instruction going on in the laboratories. As a rule, these special lectures are given by investigators, who undertake not only to *review* the field, but also *to set forth the results of their own work*. While these lectures may show the general drift of the authors' investigations, they cannot, of course, be expected to give a complete account of the facts on which the conclusions are based. In lectures of this kind due allowance must be made for the authors' limitations in time, as the subject is often one which would require a dozen or more lectures for its complete elaboration. Somewhat greater freedom in the expression of opinion than might be expected in strictly scientific communications, must also be permitted. In fact, it is one of the leading objects of this course of lectures to bring forward the *unsettled* problems of the day, and to discuss them freely. It is to be expected, of course, that now and then opposed standpoints will be developed, as has happened this time; but these differences exist, and will continue to exist, as long as anything remains for investigation, and the scientific reader will not be surprised to find them here. It may be hardly necessary to add that the authors are severally responsible for the method and form of their lectures. A brief account of the work and aims of the Laboratory will be found in the appendix.

C. O. Whitman.

[1] The first volume appeared in 1890.

CONTENTS.

LECTURE		PAGE
I.	*The Mosaic Theory of Development.* E. B. Wilson .	1
II.	*The Fertilization of the Ovum.* E. G. Conklin .	15
III.	*On Some Facts and Principles of Physiological Morphology.* J. Loeb	37
IV.	*Dynamics in Evolution.* J. A. Ryder	63
V.	*On the Nature of Cell Organization.* S. Watasé .	83
VI.	*The Inadequacy of the Cell-Theory of Development.* C. O. Whitman	105
VII.	*Balellostoma Dombeyi, Lac.* Howard Ayers . .	125
VIII.	*The Influence of External Conditions on Plant Life.* W. P. Wilson	163
IX.	*Irrito-Contractility in Plants.* J. Muirhead Macfarlane	185
X.	*The Marine Biological Stations of Europe.* Bashford Dean	211
	Appendix — *The Work and the Aims of the Marine Biological Laboratory.* C. O. Whitman.	235

FIRST LECTURE.

THE MOSAIC THEORY OF DEVELOPMENT.

EDMUND B. WILSON.

A REMARKABLE awakening of interest and change of opinion has of late taken place among working embryologists in regard to the cleavage of the ovum. So long as the study of embryology was dominated by the so-called biogenetic law, so long as the main motive of investigation was the search for phyletic relationships and the construction of systems of classification, the earlier stages of development were little heeded. The two-layered gastrula was for the most part taken as the real starting-point for research, and the segmentation stages were briefly dismissed as having little purport for the more serious problems involved in the investigation of later stages. The cleavage is equal or unequal, total or partial, regular or irregular; the diblastic condition attained by delamination, migration or invagination; the gastrulation embolic or epibolic: — such were the general conclusions announced regarding the prægastrular stages in a large proportion of the embryological papers published down to the time of Balfour and even later. The last decade has, however, witnessed so extraordinary a change of front on this subject that it will not be out of place to review briefly the three leading causes by which it has been brought to pass.

First, it has become more and more clear that the germ-layer theory is, to a certain extent, inadequate and misleading, and that even the primary layers of the "gastrula" cannot be regarded as strictly homologous throughout the animal kingdom. To assume that they are so involves us in inextricable difficulties — such as those for instance encountered in the comparison of the annelid gastrula with that of the chordates, or the com-

parison of the sexual and asexual modes of development in tunicates, bryozoa, worms and cœlenterates. This consideration led some morphologists to insist on the need of a more precise investigation of the præ-gastrular stages, and the desirability of taking as a starting-point not the two-layered gastrula but the undivided ovum. "The 'gastrula' cannot be taken as a starting-point for the investigation of comparative organogeny unless we are certain that the two layers are everywhere homologous. Simply to assume this homology is simply to beg the question. *The relationship of the inner and outer layers in the various forms of gastrulas must be investigated not only by determining their relationship to the adult body, but also by tracing out the cell-lineage or cytogeny of the individual blastomeres from the beginning of development.*"

The second of the causes referred to was the discovery of the so-called pro-morphological relations of the segmenting ovum. It is now just ten years since Roux and Pflüger independently announced the discovery that the first plane of cleavage in the frog's egg coincides with the median plane of the adult body (a fact announced many years earlier by Newport, whose observation fell, however, into oblivion). The same result was soon afterwards reached in the case of the cephalopod (Watase) and tunicate (Van Benden and Julin), and for a time it seemed not improbable that a general law had been determined. Later researches disappointed this expectation; for it was demonstrated that the first cleavage plane may be transverse to the body (annelids, gasteropods, urodeles), or even in some cases show a purely variable and inconstant relation (teleosts). The fact remained, however, that in the greater number of known cases definite relations of symmetry can be made out between the early cleavage stages and the adult body; and this fact invested these stages with a new and captivating interest.

The third and most important cause lay in the new and startling results attained by the application of experimental methods to embryological study, and especially to the investigation of cleavage. The initial impulse in this direction was given in 1883 by the investigations of Pflüger upon the influ-

ence of gravity and mechanical pressure upon the segmenting ova of the frog. These pioneer studies formed the starting-point for a series of remarkable researches by Roux, Driesch, Born and others, that have absorbed a large share of interest on the part of morphologists and physiologists alike ; and it is perhaps not too much to say that at the present day the questions raised by these experimental researches on cleavage stand foremost in the arena of biological discussion, and have for the time being thrown into the background many problems which were but yesterday generally regarded as the burning questions of the time. It is the purpose of this lecture to consider, briefly, the most central and fundamental subject of the current controversy.

It is an interesting illustration of how even scientific history repeats itself that the leading issue of to-day has many points of similarity to that raised two hundred years ago between the præ-formationists and the epigenesists. Many leading biological thinkers now find themselves compelled to accept a view that has somewhat in common with the theory of præ-formation, though differing radically from its early form as held by Bonnet and other evolutionists of the eighteenth century. No one would now maintain the archaic view that the embryo præ-exists *as such* in the ovum. Every one of its hereditary characters is, however, believed to be represented by definite structural units in the idioplasm of the germ-cell, which is therefore conceived as a kind of microcosm, not similar to, but a perfect symbol of, the macrocosm to which it gives rise (Hertwig). In its modern form this doctrine was first clearly set forth by Darwin in the theory of Pangenesis ('**68**). Twenty years later ('**89**) it was remodeled and given new life by Hugo de Vries, in a profoundly interesting treatise entitled *Intracellular Pangenesis* and in its new form was accepted by Oscar Hertwig, and pushed to its uttermost logical limit by Weismann. Kindred theories have been maintained by many other leading naturalists.

The considerations which have led to the rehabilitation of the theory of pangenesis are based upon the facts of what

Galton has called *particulate inheritance*. The phenomena of atavism, the characters of hybrids, the facts of spontaneous variation, all show that even the most minute characteristics may independently appear or disappear, may independently vary, and may independently be inherited from either parent without in any way disturbing the equilibrium of the organism, or showing any correlation with other variations. These facts, it is argued, compel the belief that hereditary characteristics are represented in the idioplasm by distinct and definite germs ("pangens," "idioblasts," "biophores," *etc.*), which may vary, appear or disappear, become active or latent, without affecting the general architecture of the substance of which they form a part. Under any other theory we must suppose variations to be caused by changes in the molecular composition of the idioplasm as a whole, and no writer has shown, even in the most approximate manner, how particulate inheritance can thus be conceived.

Based upon this conception two radically different theories of development have recently been propounded. The first of these — the so-called mosaic theory of Roux and Weismann, which forms the subject of this lecture — is based upon the assumption that the cause of differentiation lies in the nature of cell-division. Karyokinesis is conceived as qualitative in character in such wise that the idioplasmic germs are sifted apart, and cells of different prospective values receive their appropriate specific germs at the moment of their formation. The idioplasm therefore becomes progressively simpler as the ontogeny goes forward, except in the case of the germ-cells; these retain a store of the original mixture ("germ-plasm" of Weismann). Every cell must therefore possess an independent power of self-determination inherent in the specific structure of its idioplasm, and the entire ontogeny is aptly compared by Roux to a mosaic-work; it is essentially a whole arising from a number of independent self-determining parts, though Roux qualifies this conception by the admission that the self-determining power of the cell is capable in some measure, of modification, through interaction with its fellows ("correlative differentiation").

In the hands of Weismann this theory attains truly colossal proportions. The primary germs or units (which he calls "biophores") are aggregated to form "determinants," the determinants to form "ids," and the ids to form "idants," which are identified with the chromosomes of the ordinary karyokinetic figure. Upon this basis is reared a stately group of theories relating to reproduction, variation, inheritance and regeneration, which are boldly pushed to their utmost logical limit. These theories await the judgment of the future. Brilliantly elaborated and persuasively presented as they are, they do not at present, I believe, carry conviction to the minds of most naturalists, but arouse a feeling of scepticism and uncertainty; for the fine-spun thread of theory leads us little by little into an unknown region, so remote from the *terra firma* of observed fact that verification and disproof are alike impossible.

In its original form the mosaic theory has, I believe, received its death-blow from the facts of experimental embryology, though both Roux and Weismann still endeavor to maintain their position. It is rather curious that the very line of research struck out by Roux, by which he was led to the mosaic theory, should in later years have ended in a view diametrically opposed to his own. In 1888 Roux succeeded in killing (by puncture with a heated needle) one of the first two blastomeres of the segmenting frog's egg. The uninjured blastomere continued its development as if still forming a part of an entire embryo, giving rise successively to a half-blastula, half-gastrula, and half-tadpole embryo, with a single medullary fold. Analogous results were reached by operation upon four-celled stages. It was this result that led Roux to compare the development to a mosaic-work, asserting that "the development of the frog-gastrula, and of the embryo immediately derived from it is, from the second cleavage onward, a mosaic-work, consisting of at least four vertical independently developing pieces." Roux himself, however, showed that in later stages the missing half (or fourth) is perfectly restored by a process of "post-generation," which begins about the time of the formation of the medullary folds — a result which, in itself,

really contradicts the mosaic hypothesis ; for the course of events in the uninjured blastomere, or its products, is radically altered by changes on the other side of the embryo.

A more decisive result was reached in 1891 by Driesch, who succeeded, in the case of *Echinus*, in effecting a complete separation of the blastomeres by shaking them apart. A blastomere of the 2-celled stage, thus isolated, gave rise to a perfect but half-sized blastula, gastrula, and Pluteus larva ; an isolated blastomere of the 4-celled stage produced a perfect dwarf gastrula one-fourth the normal size. Even in this case, however, the earliest stages of development (cleavage) showed traces of the normal development, the isolated blastomere segmenting, as if it were a half-embryo, and only becoming a perfect whole in the blastula stage. In the following year, however, the writer repeated Driesch's experiments in the case of *Amphioxus* (the egg of which is extremely favorable for experiment), and found that in this case there is, as a rule, no preliminary half-development whatever. The isolated blastomere behaves from the beginning like an entire ovum of one-half or one-fourth the normal size.

It is quite clear that in *Amphioxus* the first two divisions of the ovum are not qualitative, as the mosaic theory assumes, but purely quantitative ; for the fact that each of the two or four blastomeres may give rise to a perfect gastrula proves that all contain the same materials. Nevertheless, in the normal development, these cells give rise to different structures — *i. e.*, they have a different prospective value — from which it follows that, in this case at least, differentiation is not caused by qualitative cell-division, but by the conditions under which the cell develops.

These facts are obviously a serious blow to the mosaic theory, and the efforts of Roux and Weismann to sustain their hypothesis in the face of such evidence only serve to emphasize the weakness of their case. In order to explain the facts of post-generation — *i. e.*, the capability of isolated blastomeres to produce complete embryos — both Roux and Weismann are compelled to set up a subsidiary hypothesis, assuming that during cell-division each cell may receive, in addition to its

specific form of idioplasm, a portion of unmodified idioplasm afforded by purely quantitative division. This unmodified idioplasm ("accessory idioplasm" of Weismann, or in some cases "germ-plasm"; "post-generation or regeneration idioplasm" of Roux) remains latent in normal development which is controlled by the active specific idioplasm. Injury to the ovum — *e. g.*, mechanical separation of the blastomeres — acts as a stimulus to the latent idioplasm, which thereupon becomes active, and causes a repetition of the original development. By assuming a variable latent period following the stimulus, Roux is able to explain the fact that regeneration takes place at different periods in different animals.

Considered as a purely formal explanation this subsidiary hypothesis is perfectly logical and complete. A little reflection will show, however, that it really abandons the entire mosaic position, by rendering the assumption of qualitative division superfluous; and, aside from this, its forced and artificial character, places a strain upon the mosaic theory under which it breaks down. Both of the two fundamental postulates of the modified theory— *viz.*, qualitative nuclear division, and accessory latent idioplasm — are purely imaginary. They are complicated assumptions in regard to phenomena of which we are really quite ignorant, and they lie at present beyond the reach of investigation. The "explanation" is, therefore, unreal; it carries no conviction, and no real explanation will be possible until we possess more certain knowledge regarding the seat of the idioplasm (which is entirely an open question), and its internal composition and mode of action (which is wholly unknown). In the meantime we certainly are not bound to accept an artificial explanation like that of Roux, however logical and complete, unless it can be shown that the phenomena are not conceivable in any other way.

We turn now to a brief consideration of opposing views, among which I ask attention especially to those of Driesch and Hertwig. In common with Kölliker and many other eminent authorities, these authors insist that cell-division is not qualitative but quantitative only, and hence is not, *per se*, a cause

of differentiation, for there is no sifting apart of the idioplasmic units, but an equal distribution of them to all the cells of the body. In other words, the cleavage of the ovum does not effect an analysis of the idioplasm into its constituent elements, but only breaks it up into a large number of similar masses. Differentiation follows upon cell-division, is caused by the interaction of the parts of the embryo, and the character of the individual cell is determined by its environment — *i. e.*, by its relation to the whole of which it forms a part. "The egg," says Hertwig, "is an organism, which multiplies by division to form numerous organisms equivalent to itself, and it is through the interactions of all these elementary organisms, at every stage of the development, that the embryo, as a whole, undergoes progressive differentiation. The development of a living creature is therefore in no wise a mosaic work, but, on the contrary, all the individual parts develop in constant relation one to another, and the development of the part is always dependent on the development of the whole." There is therefore no necessary relation between the individual blastomeres of the segmenting ovum and the parts of the adult body to which they give rise ; this relation is purely fortuitous. The most extreme statement of this view appears in the writings of Pflüger and Driesch. "I would accordingly conceive," says Pflüger, "that the fertilized egg has no more essential relation to the later organization of the animal than the snowflake has to the size and form of the avalanche which, under appropriate conditions, may develop out of it." Driesch, writing ten years later ('89), is no less explicit. He regards the blastomeres of the *Echinus* embryo, as "composed of an indifferent material, so that they may be thrown about at will, like balls in a pile, without the least impairment of their power of development." The ultimate fate of any particular blastomere is determined by its relative position in the mass ; that is (to quote his own striking aphorism), "their prospective value (*Bedeutung*) is a function of their location" (cf. His).

We shall presently return to these more extreme views, but I will here point out one all-important point which is definitely established by the work of Driesch and other experimentalists,

and which is accepted by all opponents of the mosaic theory, namely, that the cell cannot be regarded as an isolated and independent unit. The only real unity is that of the entire organism, and as long as its cells remain in continuity they are to be regarded, not as morphological individuals, but as specialized centres of action into which the living body resolves itself, and by means of which the physiological division of labor is effected. This view, at which a number of embryologists have independently arrived, has been most ably urged by Whitman, in one of the lectures of this volume, though in connection with a general conception of development peculiarly his own.

It is important not to lose sight of the fact that Hertwig, no less than Roux and Weismann, conceives the idioplasm (which he would locate in the cell-nucleus) as an aggregate of units ("idioblasts") which severally correspond to the hereditary qualities of the organism; and since cell-division is not qualitative, every cell must contain the sum total of the hereditary character of the species. Differentiation is conceived by Hertwig (following de Vries) as the result of physiological changes in the idioblasts, some of which remain latent, while others become active, and thus determine the specific character of the cell, according to the nature of the active idioblasts. In regeneration such of the latent idioblasts are called into action as are necessary to carry out the regenerative process.

We have found good reason for the conclusion that the mosaic theory cannot, in its extreme form, be maintained. It remains to inquire whether the extreme anti-mosaic conception rests upon a more secure foundation, and whether the mosaic hypothesis may not contain certain elements of truth. I have elsewhere more than once pointed out that the views of Hertwig and Driesch have received a strong bias, from the circumstance that the discussion has hitherto been confined mainly to the echinoderm egg, which shows no visible differentiation in the cells until a relatively late period (16-celled stage).

The whole question assumes a somewhat different aspect when we regard such highly differentiated types of cleavage as

we find, for example, among the annelids ; and I would ask attention for a moment to the case of *Nereis*, which is, at present, the best known form. Differentiation here begins at the very first cleavage (which is conspicuously unequal), and it becomes more pronounced with every succeeding division. The median plane is marked out at the second cleavage ; at the third the entire ectoblast of the trochal and præ-trochal regions is formed ; at the fourth the material for the entire "ventral plate" (including the ventral nerve-cord and the seta-sacs) is segregated in a single cell, that for the stomodæum in three cells ; the fifth cleavage completes the ectoblast, and by the 38-celled stage the germ-layers are completely segregated (the mesoblast in a single cell) and the architecture of the embryo is fully outlined in the arrangement of the parent blastomeres, or protoblasts.

We do not know whether, in this case, the first two blastomeres are qualitatively different, though there may be some ground for holding that they are, from the fact that the larger of the two contains a relatively larger proportion of protoplasm than the smaller.[1] But in any case their difference in size renders it impossible that they should play interchangeable parts in the cleavage. The entire later development is, however, moulded upon the 2-celled stage, every blastomere having a definite relation to it and a definite morphological value. The development is a visible mosaic-work, not one ideally conceived by a mental projection of the adult characteristics back upon the cleavage stages. The principle of "organbildende Keimbezirke" has here a real meaning and value, and this would remain true even if it should hereafter be shown that both of the first two blastomeres of *Nereis*, if isolated, could produce a perfect embryo.

It is clear, from such a case, that the more extreme views of Driesch and Hertwig cannot be accepted without considerable modification. It seems to me, however, that they may be modified in such a way as, without sacrificing the principle of epigenesis for which they contend, to recognize certain ele-

[1] All my attempts to separate these blastomeres by shaking have thus far been unsuccessful.

ments of truth in the mosaic hypothesis ; and I will attempt to indicate this modification by a comparison between *Amphioxus* and *Nereis*. In the case of *Amphioxus* we have the clearest evidence that differentiation is, in a measure, dependent upon the relation of the cell to the whole of which it forms a part. The first visible differentiation in this case is at the third cleavage, which consists in an unequal division of each of the four blastomeres, so as to give rise to four micromeres and four macromeres, the former giving rise to ectoblast only, while the latter give rise to entoblast and mesoblast as well (*Diagram* I). If, however, the blastomeres of the 4-celled stage be separated (shaken apart) the course of events is entirely changed ; for in this case each divides equally, not unequally, and ultimately gives rise to a complete quarter-sized dwarf, instead of one-quarter of a normal embryo, as it would have done under ordinary circumstances. The character of the fourth cleavage is here directly or indirectly determined in each cell by the relation of the cell to its fellows ; and if this is true of any one stage of the ontogeny, a very strong presumption is created that it is true of all — that, in the process of progressive differentiation occurring in the course of every animal ontogeny, the character of each step is determined by the condition of the entire organism. The ontogeny is, in other words, a connected series of interactions between the various parts of the embryo, in which each step establishes new relations, through which the following step is determined. The character of the series, as a whole, depends upon the first step, and this in turn upon the constitution of the original ovum. In *Amphioxus* differentiation proceeds slowly, the earlier blastomeres show no appreciable divergence, and the first stages show no trace of a mosaic work. In *Nereis*, on the other hand, a mosaic-like character appears from the beginning, because of the inequality of the first cleavage, which conditions the entire subsequent development through the

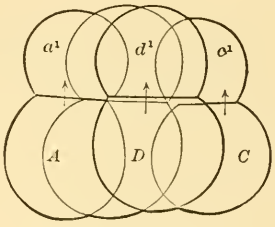


DIAGRAM I.

peculiar relations established by it. The cause of the inequality must lie in the undivided ovum, and a study of the first cleavage-spindle shows that the inequality is unmistakably foreshadowed before the least outward sign of division appears ; for the asters at the spindle-poles are conspicuously unequal in size, the larger aster corresponding with the future larger cell (Diagram II). This difference is not connected with any determinable mechanical conditions ; for the centrosomes lie nearly equidistant from the membrane (the egg is spherical), and the deutoplasm shows no perceptible inequality in horizontal distribution. The conclusion seems unavoidable that the differentiation in size is caused by a specific form of activity in the cytoplasm (or archoplasm), occurring prior to cell-division. But if a differentiation in size may have such an origin, we may fairly argue that other differentiations may likewise precede cell-division, and that in such cases the division may be, in a sense, qualitative.

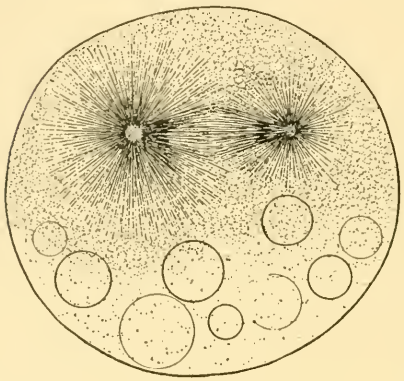


DIAGRAM II.

It seems to me, that in these considerations we may find, in some measure, a reconciliation between the extremes of both the rival theories under discussion — that we may consistently hold with Driesch that the prospective value of a cell may be a function of its location, and at the same time hold with Roux that the cell has, in some measure, an independent power of self-determination due to its inherent specific structure. Such a view is only possible, however, if we regard the specific structure of the cell to have arisen not through the segregation and isolation within its boundaries of special idioblasts or germ-substances, that have been sifted out by qualitative division, but through a physiological specialization (as de Vries and Hertwig insist) that may have taken place before, during, or after cell-division, according to circumstances. If differentia-

tion precedes or accompanies division, the latter process may be in a sense qualitative. If it follows, division will be purely quantitative, and in such a case we may rightly speak of differentiation as a result of cellular interaction. The segmentation of the egg presents more or less of a mosaic-like character, according to the period at which differentiation appears, and the rate at which it proceeds, as expressed in limitations of the power of development in the individual blastomeres, and their differences in size and structure.

The general interpretation of development which I have thus endeavored to sketch will be found to differ widely in some respects from that set forth in one of the subsequent lectures of this volume, from which, through Professor Whitman's courtesy, I am enabled to quote. Whitman argues that "cell-orientation may enable us to infer organization, but to regard it as a measure of organization is a serious error." "The question as to the presence of organization," he says, "is not settled by the *form* of cleavage. Eggs that admit of complete orientation at the first or second cleavage, or even before cleavage begins, are commonly supposed to reflect *precociously* the later organization, while eggs in which such early orientation is impossible are supposed to be more or less completely isotropic and destitute of organization. When the region of apical growth is represented by conspicuous teloblasts, the fate of which is seen to be definitely fixed from the moment of their appearance, we find it impossible to doubt the evidence of organization, or 'precocious differentiation' as it is conventionally called. When the same region is composed of more numerous cells, among which we are unable to distinguish special proliferating cells, we lapse into the irrational conviction that the absence of definitely orientable cells means just so much less organization."

It would be manifestly out of place to enter here upon any of the interesting discussions suggested by the passage just quoted, and I will therefore only add that Professor Whitman's position seems to me to rest upon a special and peculiar use of the word "organization," and that his view leads to a denial of the principle of epigenesis. No one would maintain that

the living egg is "destitute of organization," but neither can any one maintain that the egg-organization is identical with that of the adult. Development is essentially a transformation of one form of organization into another along the path of cell-division and cell-differentiation ; and it is undeniable that the adult form of organization is thus expressed earlier in some cases than in others — for example, in the segregation of the germ-layers in the polyclade, as compared with the annelid or gasteropod. We are still profoundly ignorant of the nature and causes of differentiation, and of its precise relation to cell-formation ; and the question is probably not yet ripe for discussion. It is, however, impossible to maintain that differentiation in the Metazoa is entirely independent of cell-formation, when we recall the multitude of cases in which the lines of differentiation coincide with cell-boundaries.

SECOND LECTURE.

THE FERTILIZATION OF THE OVUM.

E. G. CONKLIN.

In the history of the biological sciences perhaps no problem has received more attention than the fertilization of the ovum. The first important advance in our knowledge of this phenomenon within recent years was made by O. Hertwig[1] about twenty years ago. He showed that after a period of preparation in which the egg cell extrudes two small corpuscles, the polar bodies, the nuclei of the male and female cells fuse to form the first or segmentation nucleus of the new organism. He therefore held that the process of fertilization consisted essentially in the fusion of two nuclei coming from different individuals.

This idea has dominated biology for the past decade, and it is the generally accepted view to-day. In his recent work, *Die Zelle und die Gewebe*, Hertwig says (p. 220), "The nuclear substances which are derived in equal quantities from two different individuals, are the only essential substances upon whose union the act of fertilization depends ; they are the real fertilization materials. All other substances, such as protoplasm, yolk, nuclear sap, etc., have nothing to do with fertilization as such." It is well known that Weismann in his various essays and works upon heredity has expressed himself as fully satisfied that the essence of fertilization consists in the fusion of the nuclei of the ovum and spermatozoön. In fact his whole theory is to a very large extent founded upon this fundamental assumption that fertilization is essentially a nuclear process. And quite recently Boveri,[2] after a full discussion of the ques-

[1] O. Hertwig, Beiträge zur Kenntniss der Bildung, Befruchtung und Theilung des thierischen Eies. *Morphol. Jahrbücher*, 1875, 1877, 1878.

[2] Boveri, Befruchtung. *Ergebnisse der Anatomie u. Entwick.*, 1892.

tion, concludes with these words :[1] "Their union [that of the egg and sperm nucleus] is not the condition but the goal of fertilization, and in this sense the statement is true to-day in which O. Hertwig summed up the results of his first fundamental investigations, that '*the essential thing in fertilization is the union of egg and sperm nucleus.*'"

It is only within the most recent times that a different view has arisen, and for the sake of clearness I will sketch briefly the rise of the idea that fertilization is not a purely nuclear phenomenon, that it does not consist simply in a union of nuclei coming from different individuals, but rather, as it seems to me, in a union of *all* the essential parts of the reproductive cells, cytoplasm as well as nuclei.

In 1873 Hermann Fol[2] described in the eggs of a jelly-fish two star-shaped figures or *asters* at the two poles of the nucleus, and one year later E. Van Beneden[3] described a *polar corpuscle* as present in the cytoplasm during the karyokinetic division of the nucleus in some small parasitic organisms, the Dicyemidae ; but it was certainly not until a much later date that anything satisfactory was known of these bodies.

In 1887, only six years ago, Van Beneden[4] in his work on the fertilization of the egg of Ascaris, a thread-worm inhabiting the intestine of the horse, gave a very minute account of two granular bodies, the *sphères attractives* which he believed to be present at all times in the cytoplasm of the cell. He described each sphere as consisting of a central refractive body, the *central corpuscle*, around which was a clear space, the *medullary zone*, which in turn was surrounded by a deeply staining granular area, the *cortical zone*, Fig. 1, A and C. Although he regarded these spheres as permanent organs of the cell, he did not know how they originated in the egg undergoing fertilization, but he thought that the two spheres appeared simultaneously in the egg as newly formed structures, and he

[1] Loc. cit., p. 433.

[2] Hermann Fol, Die erste Entwicklung des Geryonideneies. *Jenaische Zeitschrift*, 1873.

[3] E. Van Beneden, Recherches sur les Dicyémides. *Bull. Acad. roy. Belg.*, 1874.

[4] Van Beneden et Neyt, Nouvelles Recherches sur la Fécondation et la Division mitosique chez l'Ascaride mégalocéphale.

believed that they were derived "from the division of the egg nucleus after it had given rise to the second polar body" (p. 60). As described by Van Beneden, the position of the attraction spheres at their earliest appearance, is shown in Fig. 1 A. They appear together in close proximity to the female pronucleus and at a considerable distance from the male pronucleus. Van Beneden therefore supposed that the male pronucleus had nothing to do with their formation. In later stages, Fig. 1, B and C, the two spheres begin to separate, the spindle fibres appearing between their central corpuscles and at the same time the two pronuclei approach each other and the first cleavage spindle is formed, in large part at least, from the two attraction spheres.

One year later, 1888, Boveri[1] described these bodies in the same egg, that of Ascaris; the central corpuscle of Van Beneden he called the *centrosome*, and the dark granular substance surrounding this, which probably corresponds to the cortical zone of Van Beneden, he named the *archoplasm*. The relation of these parts to each other, and to the pronuclei, is shown in Fig. 1, D, E and F, which are taken from Boveri. When first seen in the ovum, the male pronucleus lies in the midst of a mass of granular material, the archoplasm. Later it moves out of this, and in the place where it first lay a single highly refractive body, the centrosome, appears. The centrosome is at first single but later it divides, as shown in E, and then around each of the centrosomes the archoplasm aggregates to form two granular spheres. Meanwhile the two pronuclei enlarge greatly and approach each other while the spindle fibres are formed from the two spheres of archoplasm. With regard to the origin of the constituents of the archoplasmic system, Boveri believed that the centrosome was derived from the spermatozoön, since it first appears in the archoplasm at the very place previously occupied by the male pronucleus, while the archoplasm itself, he supposed, came entirely or at least in large part from the egg cell.[2]

[1] Boveri, Zellen-Studien, Heft 2, Die Befruchtung und Theilung des Eies von Ascaris megalocephala, 1888.

[2] Loc. cit., p. 167.

Two years ago Hermann Fol [1] published a preliminary account of the fertilization of the egg of a sea-urchin, in which he communicated the new and remarkable fact that each of the sexual cells, ovum as well as spermatozoön, contains a kinetic center or centrosome, which he called respectively the *ovocenter* and the *spermocenter*. When the spermatozoön enters the ovum the spermocenter precedes the nucleus of the male cell, Fig. 1 G, and finally reaches a point on the surface of the female pronucleus opposite the ovocenter ; then the male pronucleus comes to lie closely against the surface of the female pronucleus, though it does not intimately fuse with it. The two centers then divide, Fig. 1 H, and the half-centers pushing apart move in opposite directions around the cleavage nucleus until one half of the ovocenter meets half of the spermocenter at a point about 90° from that originally occupied by the centers, Fig. 1 I. By the fusion of these half-centres, one derived from the ovocenter the other from the spermocenter, the *astrocenters* are formed. Around each of these astrocenters there is formed a clear space, the *astrocoel*, which probably corresponds to the medullary zone of Van Beneden, as the astrocenter corresponds to the central corpuscle, while the astrocoel is surrounded by a zone of *astral radiations*, which is probably the cortical zone of Van Beneden and the archoplasm of Boveri. From these facts regarding the formation of the astrocenters, Fol concluded that "fertilization consists not only in the adding together of two pronuclei derived from individuals of different sexes, but also in the fusion of four half-centers derived from the father and the mother into two new bodies, the astrocenters." [2]

In November of the same year, 1891, Guignard [3] published a most admirable account of the process of fertilization in some of the flowering plants. He showed that the male pronucleus, which is brought into close proximity to the female pronucleus by the growth of the pollen tube, is always preceded by two

[1] Hermann Fol, Die Centrenquadrille eine neue Episode aus der Befruchtungsgeschichte. *Anat. Anzeiger*, May, 1891.

[2] Loc. cit., p. 274.

[3] Guignard, Nouvelles Études sur la Fécondation. *Annales des sciences natur.* Tom. 14, *Botanique*, 1891.

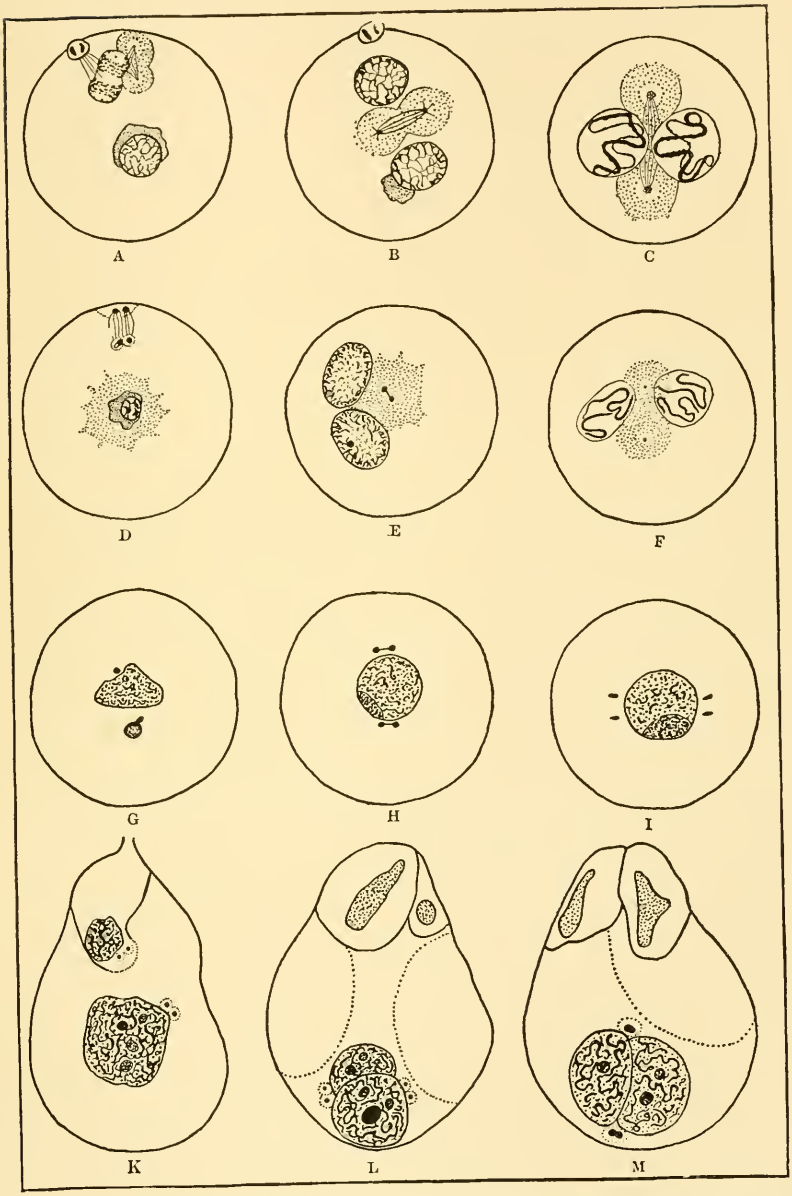


FIG. 1.— Diagrams showing the process of fertilization according to different authors. The first row (A, B and C), for Ascaris, according to Van Beneden; the second row, the same form, according to Boveri; the third row for an Echinid, according to Fol; the fourth for Lilium, according to Guignard.

bodies, the *sphères directrices*, which are the equivalents of Van Beneden's *sphères attractives* and Fol's asters; two of these spheres are also found in contact with the female pronucleus, Fig. 1 K. The two spheres which precede the male pronucleus join those which surmount the female pronucleus in such a way as to form two couples, each of which is composed of one element derived from the male cell, the other from the female cell. When the pronuclei come in contact, the two couples diverge until they come to lie at opposite poles of the nuclei, Fig. 1 L. Then the elements of each couple fuse together into a single sphere with a single central corpuscle, Fig. 1 M, and from these spheres the first cleavage spindle is formed. Guignard concludes, therefore, that fertilization is not a purely nuclear phenomenon. "It consists not only in the union of two nuclei of different sexual origin, but also in the fusion of two protoplasmic bodies whose essential elements are the *sphères directrices* of the male cell and of the female cell. Even if the nuclei are of great importance in the transmission of hereditary properties, the permanent presence of *sphères directrices* in the sexual and somatic cells, and above all their fusion at the moment of fecundation, oblige us to assign to the protoplasm the primordial rôle in the accomplishment of this phenomenon. This fusion appertains to the very essence of fertilization; it is necessary for the formation and subsequent evolution of the egg." [1]

More than a year ago (July, 1892), I found that the eggs of one of our marine Gasteropods, *Crepidula plana*, offered exceptional advantages for the study of the phenomena of fertilization. In this egg, and that of other species of the same genus, I had been able to trace the cell-lineage to more than one hundred cells, and last summer, with more favorable material and better methods, I was able to follow in surface preparations, as well as in sections, the movements of the male and female pronuclei, and what is much more important, the whole history of the *asters* [2] from the time the first polar body is formed and the

[1] Loc. cit., p. 276.

[2] For these permanent organs of the cell, known by various authors as the "*sphères attractives*," "*sphères directrices*," "archoplasmic bodies," "periplasts,"

spermatozoön enters the egg until a late stage in the cleavage of the fertilized ovum. In the main, my observations on the fertilization confirm those of Fol, concerning which a good deal of doubt has been expressed by some authorities, though in some respects they differ from these and resemble more closely the results obtained by Guignard.

Since the aster plays so important a part in the process of fertilization, it will be well to begin with a description of this structure. Every aster undergoes periodical changes in form and size as well as constitution. When it has reached its largest size, which is just before it divides, it is a spherical or ellipsoidal body, almost as large as the nucleus, Fig. 2. It has a very definite outline from which radiating rows of granules or microsomes can be traced to all parts of the cell. The sphere itself consists of an outer, darkly granular zone and of a central clear area. The granules of the outer zone can in no way be distinguished from the microsomes scattered throughout the cell; their general appearance is very similar and they stain in the same way. Moreover, at certain stages in the history of the aster it loses its definite outline, *astral radiations* proceed from it in every direction, and the granules of which it is composed become confluent with the microsomes

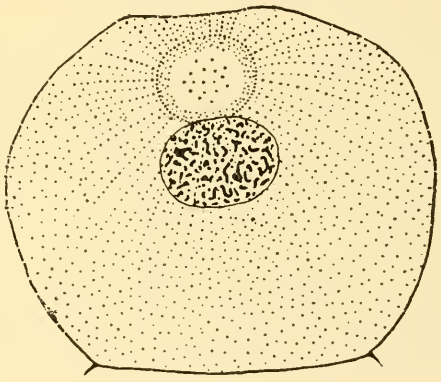


FIG. 2. — Section of one of the first four micromeres of *C. plana* showing the structure of the aster and its relations to other parts of the cell.

"para-nuclei," etc., I have decided to employ the name "aster," first used by Fol, I believe. This name has the advantage of brevity, simplicity and accuracy, which cannot be said of the others. Some of these names are not only unwieldy, but absolutely misleading. For example, the *sphères attractives* have the function of repulsion as well as attraction; the *sphères directrices* do not, in all cases at least, direct the nuclear division, and the same may be said of the archoplasm or "controlling" plasm. The exact connotation of the words "periplast" and "paranucleus" is so doubtful that they cannot safely be used.

of the cell; at the same time the central clear area disappears. This occurs in the formation of every nuclear spindle; in the later stages of the nuclear division, when the central portion of the spindle begins to disappear, the granules which were distributed along the radiating fibres are gathered together in such a way that one or more rows of them are pressed closely together to form the definite boundary of the aster, while within this boundary the granules are less compactly arranged. This *granular zone*, with its radiations, corresponds, I believe, to the cortical zone of Van Beneden and the archoplasm of Boveri.

The central clear area probably corresponds to the medullary zone of Van Beneden and the astrocoel of Fol, and in speaking of it hereafter I shall employ the latter name. The *astrocoel* always contains a large number of irregularly scattered granules, which are considerably larger than those of the outer zone but are not peculiar in any other respect. They show the same micro-chemical reactions as the microsomes, and at an early stage in the history of each aster they are closely connected with the granules of the outer zone. After the nuclear spindle has been formed these granules disappear, and in their place is found, at each pole of the spindle, a darkly staining body much larger than any one of the central granules. This is doubtless the central corpuscle of Van Beneden or centrosome of Boveri, and I cannot doubt that the numerous central granules represent a fragmented or scattered *centrosome*.

The structure of the outer granular zone of the aster, its micro-chemical reactions, and particularly its method of growth, seem to me to indicate plainly that this portion of the aster is merely a part of the general cytoplasm temporarily modified or differentiated for a particular function. Regarding the astrocoel and centrosomes there is more doubt, but I believe that the evidence is clearly in favor of the view recently expressed by Watase,[1] that these structures are also a part of the cytoplasm. Whatever the ultimate origin of the centrosomes may be, there is no doubt that in Crepidula they

[1] Watase, Homology of the Centrosome. *Jour. Morph.*, Vol. 8, No. 2.

do not go back into the nucleus after every division, as Brauer[1] asserts is the case in Ascaris. It therefore seems highly probable that the entire aster is a cytoplasmic structure, temporarily modified or differentiated for the purpose of contraction and expansion, whose chief function in the reproductive cells is to bring the pro-nuclei together, accomplish nuclear division, and move the nuclei from place to place in the resulting cells.

To return to the process of fertilization : the spermatozoön usually, though not invariably, enters the ovum near the vegetal pole. I have not observed it in the actual process of entering, though I have seen it immediately afterward. In such cases it consists of a small conical or sometimes fusiform nucleus, surrounded by a clear non-granular area, Fig. 3. At this stage the sperm nucleus consists entirely of chromatin closely packed together; there is no nuclear sap and no aster is visible, though, perhaps, the clear area surrounding the nucleus may be taken as an indication of the presence of such a body. In Fig. 3 the egg nucleus is shown in the process of division preparatory to the formation of the first polar body. There are two centrosomes at the upper pole of the spindle, though but one is to be seen at the lower pole. The centrosome at the upper pole has divided thus early, preparatory to the division of the chromatin of the first polar body. This division occurs soon after the polar body is formed. The surface of the egg is indented over the upper pole of the spindle, thus indicating, as it seems to me, that the astral fibres are here attached to the surface and are drawing it in by their contraction.

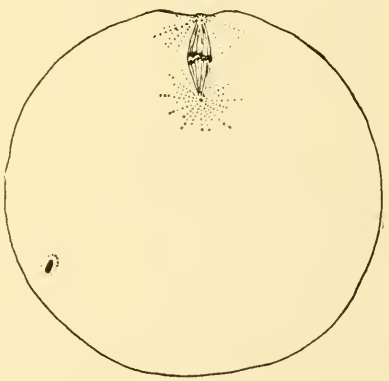


FIG. 3. — *C. plana ;* formation of the first polar body ; at the left the spermatozoön has just entered the ovum.

[1] Brauer, Zur Kenntniss der Herkunft des Centrosomes. *Biol. Centralblatt,* Bd. 13, Nos. 9 and 10.

The spermatozoön usually enters the ovum at about the same time that the first polar body is being formed, though in a considerable number of eggs it does not enter until after both polar bodies have been extruded. Soon after its entrance, the sperm nucleus begins to move toward the egg nucleus. This motion is quite slow, several hours (four to eight) being necessary to bring the two nuclei together; it is, therefore, possible to find almost every stage in this process in eggs which have been fixed and stained. During the whole of this journey toward the egg nucleus, the sperm nucleus continually increases in size; but before this nucleus shows any appreciable enlargement, and when it is removed from the egg nucleus by almost the whole diameter of the ovum, the sperm aster appears as a granular sphere, considerably larger than the sperm nucleus, and lying immediately in advance of it, Fig. 4. Throughout the approach of the two pronuclei, the sperm aster precedes the sperm nucleus, and, as I believe, actually leads it to the egg nucleus.

As already explained, Boveri believes that the centrosome of the new organism is derived exclusively from the spermatozoön. In his latest work (already referred to) he says that, in the case of Ascaris, there is neither centrosome nor aster present in the formation of the two polar bodies, although they were present at an earlier stage in the oögenesis; he therefore concludes that in this case the centrosomes of the segmentation spindle, which forms later, must be derived exclusively from the sperm centrosome. Vejdovsky[1] has reached the same conclusion with regard to one of the annelids, Rhinchelmis. He says, regarding Fol's communication relative to the ovocenter, that no one has hitherto seen such a body in connection with the female pronucleus, and that he conceives it to be the scarcely functional remnant of the *Eikernperiplaste*, or egg aster. "It is therefore," he says, "questionable to assume the presence of an ovocenter in order to make a general law that in fertilization not only the pronuclei, but also the halves of the ovocenter and spermo-

[1] Vejdovsky. Bemerkungen zur Mitteilung H. Fol's "Contribution à l'histoire de la fécondation." *Anat. Anzeiger*, Bd. 6, No. 13.

center unite. The facts observed in the case of Rhinchelmis speak against Fol's theory."

In the face of the conclusions of these well-known investigators, it is interesting to find, in the case of the Crepidula egg, a well marked centrosome and surrounding parts of the aster present at each pole of the spindle in the formation of each of the polar bodies; and immediately after the second polar body has been extruded, and while the sperm nucleus and aster are still far removed from the egg nucleus, a large and distinct aster can be seen in contact with this nucleus. This egg aster lies below the egg nucleus, and usually slightly to one side of the chief axis of the ovum, as shown in Fig. 4.

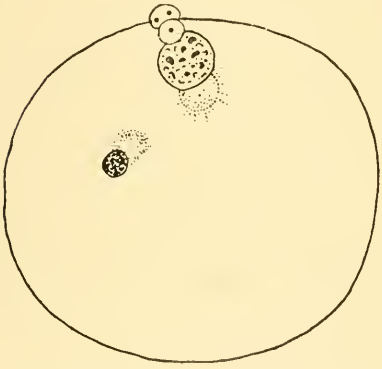


FIG. 4. — Ovum of *C. plana*, side view; the asters are shown in dotted outline, the clear spheres with dark centers, at the upper pole, are the two polar bodies.

FIG. 5.— Ovum of *C. plana* seen from upper pole; in this and the following figures the male aster and nucleus lie to the left, the female to the right. First contact of the two asters.

It is at first much larger than the sperm aster, and in fact remains larger until it enters upon "*la Marche de la Quadrille.*"

The sperm nucleus always approaches the egg nucleus from below and in such a way that the sperm aster is directed toward the egg aster, Fig. 4. The force which draws the two pronuclei together seems to exist between the two asters rather than between the pronuclei. Accordingly, in the progress of the sperm nucleus toward the egg nucleus, the asters first come in contact, Fig. 5. In all movements of the nucleus, the latter appears to be passive while the asters are active. This

principle is illustrated throughout the whole history of the cleavage ; whenever the nucleus is moved from one position to another in the cell it is preceded by its aster. The method by which the asters draw the two pronuclei together is, I believe, by the formation, attachment and contraction of the astral or archoplasmic fibrils. In this connection it is interesting to contrast the methods by which the male and female cells approach each other before and after the spermatozoön has reached the ovum. In the case of Crepidula, as in most other forms, the spermatozoön moves toward the ovum by the lashing from side to side of a long thread-like flagellum. As soon, however, as the sperm enters the ovum this flagellum is

FIG. 6. — Ovum of *C. plana* from upper pole; first contact of the two pronuclei.

FIG. 7. — Separation of the two asters; the polar bodies lie immediately over the female pronucleus.

lost in most cases, and the rest of the progress toward the egg nucleus and aster must be accomplished in some other way. This, as just said, seems to be done by the formation and contraction of astral fibers. This movement of the sperm cell within the ovum is the result of protoplasmic contractility no less than is the movement of the spermatozoön outside of of the nucleus, operating, however, in a slightly different way.

After the two asters have come in contact, they move slightly to one side, remaining however connected, though individually distinct, and the two pronuclei then come together, Fig. 6. Even at this time, the sperm aster and nucleus are usually a

little smaller than those of the egg cell, and they frequently remain slightly smaller as long as they can be distinguished. In cases where there is no appreciable difference in size between the two pronuclei, the one may be distinguished from the other, as long as they remain distinct, by the position of the polar bodies which lie directly over the female pronucleus.

After the two pronuclei have met, the two asters begin to move apart. They continue to separate, moving around the appressed nuclei, until they lie at opposite poles, Fig. 7. The sperm aster now lies on the outer side of the sperm nucleus, and the egg aster on the outer side of the egg nucleus ; it is thus seen that the position which the asters occupy relative to

FIG. 8. — The halves of the male and female asters about to unite at the poles of the pronuclei.

FIG. 9. — The halves of the female aster at the poles of the pronuclei ; the male aster still undivided.

the pronuclei, is just the reverse of that which obtained previous to the meeting of the two pronuclei. It must also be allowed, I think, that the function now exercised by the asters must be the reverse of that which prevailed before the pronuclei met. Then, by active contraction, they drew the two pronuclei together; now, by active expansion, they diverge, move to opposite poles, and press the pronuclei together. This functional alternation of contraction and expansion, or perhaps better, attraction and repulsion, is manifest not only during fertilization, but throughout the entire process of cleavage. The contraction can be seen in the way in which the asters

draw the nuclei from one place to another in the cell, as well as in the pulling asunder of the chromatic elements of the nucleus, the expansion in the division of the asters and the subsequent divergence of the resulting daughter asters, as well as in the formation of the karyokinetic spindle and the pushing together of the chromatic elements into the equatorial plate.

After the asters have reached the outer sides of the two pronuclei, each divides into two half-asters, which diverge from each other until they come to lie at opposite poles of the nuclei and in the plane of contact between the two. At this stage, therefore, there is found at each pole of the two pronuclei one-half of the sperm aster and one-half of the egg aster, Fig. 8. Each of these couples soon fuses into a single aster, and the two asters thus formed lie at opposite poles of the first cleavage-spindle, Fig. 10, and from them all the other asters of the developing ovum are derived.

As an interesting variation of this more usual behavior of the asters, it should be mentioned that in cases where the sperm nucleus and aster meet the corresponding parts of the egg cell, while there is yet considerable disparity in size between the two, the egg aster after having reached its full size divides, and the two half-asters pass to the two poles of the nuclei, while the sperm aster, during all this time, remains undivided until it has reached its full size, when it divides, and its halves move around to meet the waiting halves of the egg aster, Fig. 9.

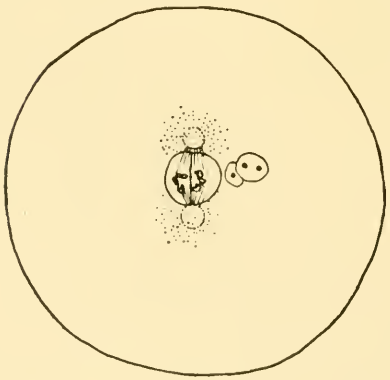


FIG. 10. — Formation of the first cleavage-spindle. The half-asters have fused, but a trace of the nuclear membrane remains between the two pronuclei.

With the fusion of the half-asters, one part of the fecundation, and that a very important one, is completed, although the two pronuclei may still be recognized as such. In fact, the boundary line between the egg and the sperm nucleus can be

distinguished even after the first cleavage spindle begins to form, and their chromatin masses remain distinct, Fig. 10, until the equatorial plate stage.

After the chromatin of the two pronuclei has assumed the shape of distinct rods, the chromosomes, and before these rods have been pressed together into the equatorial plate, it is possible to determine that each pronucleus contains twelve of these elements. Since these elements are doubled at every division of the nucleus, it follows that each of the first two nuclei contains twenty-four chromosomes, twelve derived from the sperm nucleus and twelve from the egg nucleus, and this number is probably constant for all the nuclei of this species.

It is difficult to define exactly the time at which the two pronuclei have sufficiently united to be considered a single element, but perhaps this can be most conveniently located at the time when the boundary wall between the two vesicular nuclei disappears, although, as just said, the two masses of chromatin remain distinct until a somewhat later period. Certain it is that a fusion of the pronuclei to form a resting *segmentation nucleus*, as described by Hertwig, does not take place.

With the union of the pronuclei, the entire process of fecundation may be considered as ended. So far from being a purely nuclear phenomenon, it must be evident to any one who follows the history of the asters that they take an extremely important part in this process. If we are amazed at the precision with which the chromatic elements of the nucleus are divided and distributed, we can be no less astonished at the wonderful directive influence exercised by the asters upon the nuclei; and if it is justifiable to conclude that the chromosomes have an extremely important function in the building of the new organism because of the way in which they are distributed, how is it possible to avoid the same conclusion with regard to the asters, which, as we have seen, are equally divided in a manner not clearly understood into half-asters, which then fuse in such a way that one-half of each aster of the new organism comes from the paternal and one-half from the maternal organism?

Hertwig, Boveri and Weismann, as well as a number of other well-known investigators, assert that the hereditary substance, by means of which heritable qualities are transmitted from one generation to another, is contained wholly within the nucleus, and, speaking more strictly still, within that part of the nucleus called the chromatin. Since, however, many of the characters of the cytoplasm are heritable, and in fact are generally the only characters which are known by observation to be heritable, these authorities are compelled to assume that the control of the cell, if not the actual genesis of all its constituents, is located wholly within the chromatin. As a matter of fact, it must be acknowledged that this assumption that the chromatin makes the cell-body just what it is in point of structure, function, shape, position and size, does not rest upon observation but upon supposed theoretical necessities. All these necessities find their source in the assumption that the nucleus, and especially the chromatin, is the only bearer of heredity, an assumption which was wholly justified so long as it was supposed that fertilization consisted merely in the fusion of the two pronuclei ; but now since it is known that the cytoplasm, and especially the male and female asters, take a very important part in the process of fertilization, it seems to me that such an assumption is wholly unwarrantable.

The spermatozoön does not cease to be a cell the moment it has entered the ovum. Both its cytoplasm (in the form of an aster) and its nucleus are represented, and they grow just as a cleavage or tissue cell does by the assimilation of food material, which in this case is contained within the egg cell. Although within the ovum the spermatozoön must be considered a distinct cell, preserving all its fundamental peculiarities, until that time when its various constituents lose their identity by fusion with corresponding parts of the egg cell. While it is known in several cases that the spermatozoön introduces a nucleus and an aster, which then fuse with similar parts of the ovum, it is not known, with one or two exceptions, that any portion of the general cytoplasm of the male cell is carried into the ovum. In the case of Ascaris, both Van Beneden and Boveri agree that a certain part of the cytoplasm of the spermatozoön

is carried into the egg, and is there absorbed, used as food, by the cytoplasm of the ovum. On the ground of direct observation, the fusion of the general cytoplasm of the sperm with that of the ovum can neither be affirmed nor denied in most cases ; but it is a matter of observation that there is a union of those portions of the cytoplasm which can be followed, viz., the asters, and in view of what we know of conjugation among the simplest animals and plants, where there is a fusion of the cell-bodies as well as of the nuclei, I question whether the cytoplasm which the sperm carries into the ovum, in the case of Ascaris, merely degenerates and serves as food for the egg cytoplasm. It seems to me more probable that this sperm cytoplasm does not act as so much dead matter, but that it also takes part in this union of the essential constituents of the two cells.

On *a priori* ground, I think we ought to expect that in fertilization all the essential parts of one cell would unite with corresponding parts of another cell. In fact, the form of fertilization characteristic of the higher animals and plants is generally supposed to have been derived from a condition similar to that which at present obtains among the lower animals and plants in which there is a fusion of two entire cells. Most persons would probably agree that fertilization consists in the union of the essential parts of two cells ; the question is, "What are essential parts?" It is known that if some of the Infusoria are cut to pieces so that some of the pieces contain portions of the nucleus, while others do not, the nucleated portions will regenerate all the lost parts, while those pieces which contain no part of the nucleus do not regenerate but sooner or later perish. It is, therefore, certain that the cytoplasm cannot perform the normal funtions of growth and regeneration apart from the nucleus. It seems equally certain, although much more difficult of demonstration, that the nucleus cannot perform all its normal functions apart from the cytoplasm. Both nucleus and cytoplasm are essential constituents of the cell, and one cannot be said to be more important than the other. In spite of the assumption of smaller structural units within the cell (such as the *biophors* of Weismann, the

pangenes of De Vries, the *plasomes* of Wiesner, which assumption, in one form or another, seems to me a necessity), the *independent* unit of structure is still the entire cell, not cytoplasm alone, nor nucleus alone, but the two together. So far as we know, the cytoplasm of every cell comes from the cytoplasm of some parental cell, just as certainly as the nucleus comes from a preceding nucleus. Until, therefore, it can be shown that the nucleus can exist independently of the cytoplasm, it is unsound to assert that the cytoplasm is not an essential constituent of the cell. And until some one has shown that cytoplasm is not derived from pre-existing cytoplasm, but is the product of the nucleus,[1] it is too soon to assert that the control of the entire cell lies in the nucleus, and hence that the nucleus is the sole bearer of heredity.

In seeking to show that the nucleus is not the sole bearer of heredity, we are not confined entirely to the *a priori* argument ; aside from the important evidence already adduced in the presence and function of the asters observations are not altogether wanting to show that the cytoplasm, in many respects at least, is not controlled by the nucleus. In the early cleavage stages of *Crepidula plana*, it can be shown beyond question that the *direction* of the cleavage, the *size* of the cells formed, and the *shape* of those cells is the result of cytoplasmic activity rather than of nuclear.[2] It is generally believed that

[1] This is, I know, what Weismann asserts (The Germ Plasm, p. 50), and he urges in proof the observations of Rückert on the alteration in size of the chromosomes of the nucleus during the growth of the ovum of the dogfish. These chromosomes first enlarge greatly, and afterward diminish in size until they are not much larger than at first. Rückert, therefore, believes that the chromosomes give off a large amount of substance to the cytoplasm, and this conclusion is probably correct. It does not follow, however, that this material controls the cytoplasm, as Weismann assumes, any more than the fact that the cytoplasm supplies the nucleus with a large amount of nuclear sap in its pre-division stages, argues that the cytoplasm controls the nucleus. It is probable that each supplies substances to the other, and it is interesting to note that at the very stage described by Rückert (the pre-division stage), in which the chromosomes are giving off substances to the cytoplasm, the nucleus, in many forms at least, is receiving a large amount of material in the form of nuclear sap from the cytoplasm.

[2] Since this lecture was given, I have found that Boveri, in his recent work on

the nuclear spindle predetermines the direction of the cleavage, yet, in this case, the spindle may be formed in any possible direction, and yet the cleavage invariably takes place in the same direction. It is frequently asserted that the division-wall between the daughter-cells appears at right angles to the spindle, and yet, in this case, it may form at a greater or a smaller angle than 90°. The direction of the cleavage is predetermined not only before the nucleus divides, but long before the asters divide. Certain oscillatory movements of the cytoplasm, which begin with the first division of the ovum and can be observed throughout a large part of the cleavage, determine with absolute certainty the direction of the cleavage before any indication of division can be found within the cell. Since the direction of the cleavage is not determined by the nucleus, it follows that the rotation of the cells and the position which they take, with reference to each other, are not determined by the nucleus ; and since it can be shown that the shape of the cleavage cells is very largely the result of intercellular relations, it follows that in this case, at least, the shape of the cell is not determined by the nucleus.

Still farther, the size of the cell seems to be determined by the cytoplasm rather than by the nucleus. The indirect or karyokenetic division of the nucleus results in an equal division of the chromatic substance. After the division has taken place, the two-daughter nuclei are for a considerable period equal in size, though the cell-bodies in which they lie may be very unequal. Likewise, the division of the asters is always an equal division, and in the early stages of karyokinesis the two asters are always equal in size. Yet in these very stages in which the nuclei and the asters are equal in size, the lobing of the cytoplasm may show beyond doubt that the division of the cell-body is to be very unequal. I believe, therefore, that neither the nuclei nor the asters determine the initial size of

fertilization ("Befruchtung," p. 469), mentions the fact that he fertilized ova of one genus of sea-urchin with the sperm of another, from which cross a larval form developed which was intermediate in character between the two genera. The cleavage, however, was purely maternal, thus indicating that it was not influenced by the sperm nucleus. He, therefore, concludes that the process of cleavage, to all appearances at least, is not directed by the nucleus.

the cells which are formed by division ; it is determined rather by the general cytoplasm. In the later stages of karyokinesis, after the lobing of the cytoplasm has indicated the size of the daughter cells, but sometime before the division-wall is formed, the asters become unequal in size if the division is to be an unequal one, and by the time that the division-wall is formed the asters are proportional in size to the daughter cells in which they lie. At a considerably later period the nuclei become proportional in size to the cells in which they are found.

In Crepidula, therefore, I believe it is certain that the direction of the cleavage, as also the position, the shape and the size of the resulting cells are not directly governed by the nucleus. These definite forms of cleavage which are so excellently exemplified in Crepidula are inherited as certainly as any definite adult structures are, and if they are not under nuclear control, these hereditary tendencies must be transmitted through the cytoplasm.

Of course, it may be urged that there is some unknown and invisible influence emanating from the nucleus which controls all the processes of cell life. In the nature of the case such an assertion cannot be affirmed nor denied on the ground of observation, and it seems to me sufficient to urge in reply that we should believe things are what they seem unless we are compelled to believe differently. Many of the processes of cell life seem to be controlled by the cytoplasm more intimately than by the nucleus ; so far as we can observe, all cytoplasm comes from pre-existing cytoplasm, just as all nuclei come from previous nuclei. We know that the cytoplasm from both the father and mother are represented in every stage of fertilization, and it is unnecessary and therefore unscientific to assume that the nucleus controls all the processes of cell life, and that the heritable characters of the cytoplasm are transmitted only through the nucleus.

If, in stating my objections to the view held by the vast majority of the biologists of the present day, I may seem to have denied the importance of the nucleus while emphasizing the importance of the cytoplasm, I would wish to say, in con-

clusion, what has been said several times already, that I consider both cytoplasm and nucleus essential constituents of the cell, and therefore one cannot be said to be more important than the other. In all the phenomena of cell life, — growth, regeneration, fertilization, division, the building of the organism, the transmission of heritable characters, — both nucleus and cytoplasm take part, though probably not always an equal part. The *entire* cell is still the ultimate independent unit of organic structure and function.

THIRD LECTURE.

ON SOME FACTS AND PRINCIPLES OF PHYSIOLOGICAL MORPHOLOGY.

JACQUES LOEB.

In this address I shall give a short account of a series of experiments which were undertaken in order to determine the causes of animal forms. Some of the results of these investigations have already been published.[1] Among others, which are new, are experiments upon the artificial production of double and multiple monstrosities from one ovum in the sea-urchin.

I. Heteromorphosis.

If we look at an animal we perceive that its various organs are arranged in a definite way. From our shoulders originate arms, and from our hips legs, but we never see that legs grow out from the shoulders or arms from the hips. In the lower animals there exists the same definite arrangement of parts.

Fig. 1 is a diagram of a hydroid Antennularia, which is pretty common at Naples. From a bundle of roots or stolons a perfectly straight stem arises to a height of six inches or more. From this main stem originate, in regular succession, very short and slender branches, which carry polyps on their upper sides.

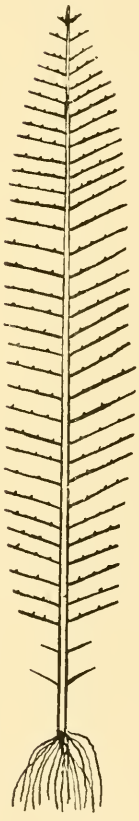


Fig. 1.

In this case we never find that a root originates at the apex, or in the place of a branch, or that polyps originate at the lower side of a branch. To the

[1] *Untersuchungen zur physiologischen Morphologie der Thiere.* I, Heteromorphosis, Würzburg, 1891. II, Organbildung und Wachsthum, Würzburg, 1892.

physiologist the question arises: What are the circumstances which determine that only one kind of organs originate at certain places in the body? I conceived that the answer to this question might be obtained by finding out whether or not it was possible to make any desired organ of an animal grow at any desired place. In case of success, the question to be decided was whether the same circumstances by which we can change the arrangement of organs experimentally determine also the arrangement of organs in the natural development.

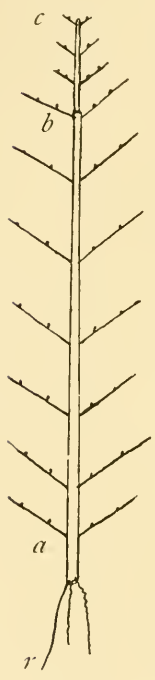


FIG. 2.

If we cut out a piece (Fig. 2) of an Antennularia, and hang it up vertically in the water, the apical end *b* above and the root end *a* below, we find that after a few days the root end *a* forms little roots, *r*, which grow downward, and the apical end, *b*, forms a new apex, *c*.

If we cut out a similar piece and hang it upside down (Fig. 3), the root end *a*, which now is above, forms a new apex, *ac*, and the apical end *b*, which is below, forms roots. In such an apex the arrangement of organs is just the same as in the normal animal, namely, branches growing obliquely upward bearing polyps on their upper sides. We are, therefore, able to substitute a root for an apex and an apex for a root. I have called this substitution of one organ for another heteromorphosis. If we place the cut-out piece of Antennularia horizontally instead of vertically, something still more remarkable happens, namely, the branches on the lower side suddenly begin to grow vertically downward, and the outgrowing parts are no longer branches but roots, *rr*, Fig. 4. This we can prove by their physiological reactions, for the roots fix themselves to the surface of solid bodies, for instance, the glass of

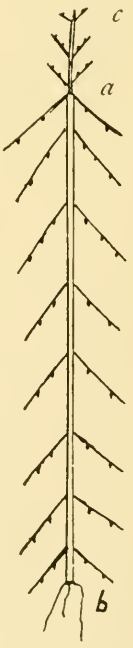


FIG. 3.

the aquarium, while the stems never show any reaction of this kind. These new parts growing out from the branches of the under side of the stem attach themselves to solid bodies, if we bring them in contact with the same. Moreover, they are positively geotropic (that is, they grow toward the centre of the earth), while the branches never show any positive geotropism. The branches on the upper side are not transformed into roots.

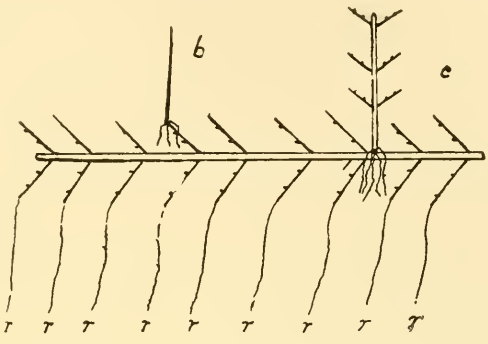


Fig. 4.

They either perish or give rise to very long, slender, perfectly straight stems (*b*, Fig. 4), which grow vertically upward. These stems, as a rule, are too slender to bear branches, but at parts of the upper surface of the main stem there originate new stems (*c*, Fig. 4) which grow vertically upward and produce the typical little branches with polyps.

If we bring the stem into an oblique position (Fig 5), with the apex *b* upward, from every element of the main stem new

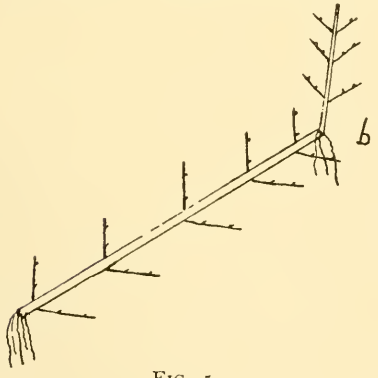


Fig. 5.

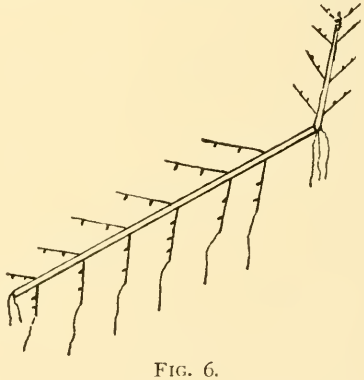


Fig. 6.

stems and roots may originate, but with this difference, stems always originate from the upper side of an element and roots from its lower side. But if we place the stem in an oblique

position (Fig. 6), with the root end above, the branches on the under side grow out as roots and at the upper end, a stem arises as usual.

What circumstances have all these experiments in common? These two : stems always originate from the upper end or side of an element, and roots always originate from the lower side or end of the same element. These facts can be explained only through the assumption that gravitation, in this case, determines the place of origin of organs.

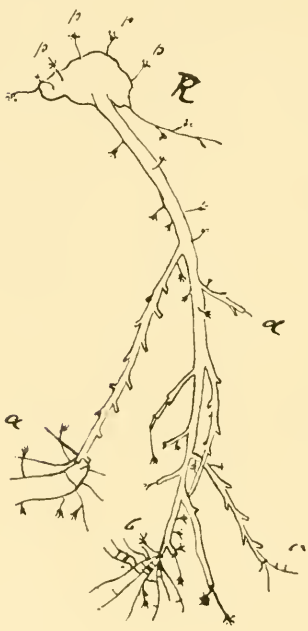


Fig. 7.

Now we may ask whether the action of this force, gravitation, produces the natural arrangements of parts, *i. e.*, roots growing only at the base of the stem and never at the apex or in the place of a branch. I think that it does. By reason of its negative geotropism, the stem grows vertically upwards. Gravitation does not permit roots to arise at any place except the under side of the organs, and that, in the normal position, is the base of the stem. The same force determines that polyps can originate only at the upper side of branches, and so the main arrangement of organs is brought about by gravitation. But how does gravitation determine that roots grow at the upper and stems at the under side? This is a question to which I shall return later.

Fig. 7 is a drawing of a case of heteromorphosis in Margeliss, a hydroid common at Woods Holl.

If we cut off a stem, or a small piece of a stem, and place it in a dish containing sea water, and protect it carefully from every motion, a curious change takes place in the organism. Almost all, and in some cases all of the stems which touch the glass begin to give rise to roots that spread out and very

soon cover a large area of the glass. In this way the apical end of a stem may continue to grow as a totally different organ, namely, as a root. Every organ not in contact with some solid body gives rise to polyps. Even the main root, if not in contact with a solid body, no longer grows as a root, but gives rise to a great number of small polyps which appear at the end of long stems. Fig. 7, which Mr. Tower was kind enough to draw for me, shows a branch which formed roots at its apex and polyps at its roots in this way. The stem touched the bottom of the dish with the apical ends, *a*, *b*, *c* and *d*. All these ends gave rise to roots. From the upper side of the original root, *r*, which was not in contact with the glass, later on small polyps, *pp*, grew out. Every place which was in contact with solid bodies gave rise to roots, and every place which was in contact with sea water gave rise to polyps.

This is not the only species of hydroid found at Woods Holl in which such forms of Heteromorphosis can be produced. Another form, Pennaria, is just as favorable. In Pennaria I succeeded repeatedly in producing roots at both ends of a small stem that bore no polyps.[1]

What circumstances are common in these experiments on Margelis and Pennaria? Organs brought into contact with solid bodies continue to grow as roots, if they grow at all. Organs surrounded on all sides by water continue to grow in the form of polyps, if they grow at all. In Margelis, contact

[1] In a Tubularian I was able to produce the opposite case, namely, to get an animal that ended at both ends in a polyp and had no root. Weismann seems to assume, in his "Germ Plasm," that the latter result is to be explained by the principle of natural selection, inasmuch as an animal without polyps could not continue to live, and hence it would be impossible to produce roots at both ends. In Pennaria this supposed impossibility was realized. One may say that these roots in Pennaria may give rise later on to polyps. In the special case that I observed they did not, although as a rule they do. But the same is the case in Tubularia, in which polyps also arise from the roots. It might be said, perhaps, that the formation of roots in Pennaria is, for some reason, absolutely necessary. But it is just as easy to produce polyps at both ends. Even if it were possible to reconcile these facts with the principles of natural selection, causal or physiological morphology would not gain thereby, as the circumstances that determine the forms of animals and plants are only the different forms of energy in the sense in which this word is used by the physicist, and have nothing to do with natural selection.

with a solid body plays the same rôle as did gravitation in the case of Antennularia. In what way the contact may have an influence shall be mentioned later. We may add, however, one more point. In Antennularia, gravitation not only determines the place of origin of the various organs, but also the direction of their growth ; the stem, growing upward, is negatively geotropic, the root, growing downward, is positively geotropic. In Pennaria, the nature of the contact not only determines the place of origin of the various organs, but also the direction of their growth. If we bring an outgrowing polyp of Pennaria into contact with a solid body, the polyp begins to grow away from the body, and the new stem is very soon nearly perpendicular to the part of the surface with which it came into contact.

I have called this form of irritability Stereotropism. We may speak of positive Stereotropism in the case of the root, and of negative Stereotropism in the case of the polyp.

Here, too, it may be asked whether contact with foreign bodies, which in these experiments determined the arrangement of the various organs, may not have the same effect in the natural development of the organism. I believe that such is the case. Negative Stereotropism forces the polyps to grow away from the ground into the water, and so parts surrounded by water form polyps only. Positive Stereotropism forces roots in contact with the ground to grow toward it, so parts in contact with the ground give rise to roots only. Thus it happens that, under ordinary circumstances, in the animal we find roots only at the base where it touches the ground. In other hydroids the place of origin of the different organs is determined by light, and in others we find more complicated relations.

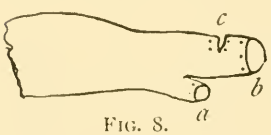


FIG. 8.

It may appear from the foregoing that such cases of heteromorphosis are confined to hydroids, but such is not the case. We find similar cases in *Tunicates*. *Ciona intestinalis* (Fig. 8), a solitary ascidian, has eye-spots around the two openings into the pharyngeal cavity, *a* and *b*. If we make an incision at *c*, eye-spots are formed on both sides of the incision.

II. Polarization.

While the foregoing experiments were in progress, I observed that in many animals I was unable to produce any kind of heteromorphism. These animals showed, in regard to the formation of organs, a phenomenon with which we are familiar in a magnet. If we break a magnet into pieces, every piece has its north pole on that side which in the unbroken magnet was directed toward the north. Likewise, there are animals every piece of which produces, at either end, that organ toward which it was directed in the normal condition. We may speak in such cases of polarization. The clearest example of this I found in an actinian, *Cerianthus membranaceus*.

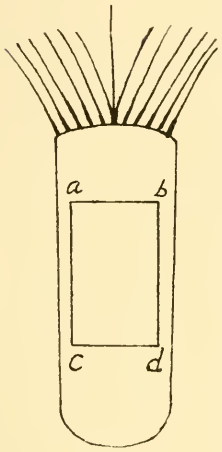


Fig. 9.

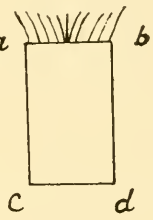


Fig. 10.

If we cut a rectangular piece, *a b c d*, out of the body-wall of Cerianthus (Fig. 9), very soon new tentacles begin to grow out of this piece, but only from the side *a b* (Fig. 10), which was directed toward the oral end of the animal. Nothing of the sort occurs in the side, *c d*, or *a c*, or *b d*. The production of tentacles takes place before any other regeneration begins. The same polarization is shown in the following variation of the preceding experiment: If we make an incision, *a b c* (Fig. 11), into the body-wall of the actinian, only the lower lip, *b c*, produces tentacles, while the upper lip, *a c*, produces none. The two ends heal together in such a way that one-half of a mouth, with its surrounding tentacles, *a* (Fig. 12), is formed. It is curious to see how these tentacles behave if

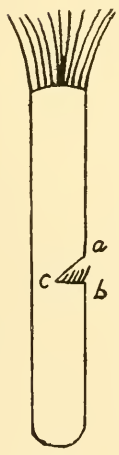


Fig. 11.

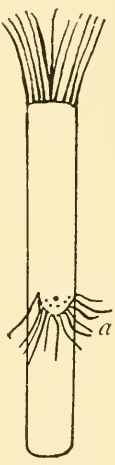


Fig. 12.

we offer them bits of meat. They endeavor to force it into the new oral disc, where the mouth ought to be, but where no mouth exists, and only after a struggle of some minutes give up the hopeless attempt. I tried in every possible way to produce tentacles in the aboral end of a piece which had been cut out, but without success.

Hydra behaves, as regards polarization, a little differently from Cerianthus. If we make an incision in the stem, a whole new oral pole grows out, but otherwise it, too, shows polarization.

A good many animals, so far as is yet known, reproduce only the lost organ, but never show any heteromorphism. We see, therefore, that while in some animals we are able to produce heteromorphosis, in others the most definite polarization exists, and we are able to produce regeneration of lost parts only in the arrangement which exists in the normal animal. In this case we must assume that unknown *internal* conditions determine the arrangement of limbs.

In addition to examples of heteromorphosis or polarization occurring separately, we find cases in which both phenomena are exhibited by the same animal. If we cut out a sufficiently large piece of the stem of Tubularia mesembryanthemum, and place it in the bottom of a dish of water, carefully protected from jarring, the anterior end of the piece gives rise to a new polyp, the posterior end to a root ; but if we hang up the stem in such a way that the posterior end does not touch the surface of the glass, and is sufficiently provided with oxygen, this end, too, produces a polyp, and we have a true case of heteromorphosis. In all cases the polyp at the oral end is formed first, and a relatively long time (one or more weeks) elapses before the aboral polyp is formed. But under one condition I could cause the stem to form a polyp at the aboral as quickly as at the oral end, namely, by inhibiting or retarding the formation of the oral polyp. This could be done readily by diminishing the supply of oxygen at the oral end. In such cases the aboral polyps were produced nearly as quickly as the oral polyps.

III. The Mechanics of Growth in Animals.

In order to get an explanation of the phenomena of organization we must ask, What are the physical forces that determine the formation of a new organ? We know that the ultimate sources of energy for all the functions of living bodies are chemical processes. The question is, How can these chemical forces be brought into relation with the visible changes which take place in the formation of a new organ? The answer to this question is to be obtained by a knowledge of the mechanics of growth. It is very remarkable that the mechanics of growth forms almost an empty page in the history of animal morphology and physiology. I can refer here only to the few experiments that I have made on this subject; but fortunately the subject has been worked out very carefully in plants, and as my experiments show that the conditions for growth in animals are, to a certain extent at least, the same as the conditions for growth in plants, we have the beginning of a basis for work.

A brief outline of the manner of growth in plants is as follows: Before the cell grows it forms substances which attract water from the surroundings, or, as the physicist expresses it, it forms substances which determine a higher osmotic pressure within the cell than did the substances from which they originate. The walls of the cell, or rather the protoplasmic layer that lines the cell wall, possesses peculiar osmotic properties, in consequence of which it allows molecules of water to pass through freely while remaining resistant to the passing through of the molecules of many salts dissolved in the water. The result is that when substances of higher osmotic pressure are formed inside the cell, water from the outside passes in until the pressure within again equals the pressure without. The cell-wall becomes stretched and, according to Traube, new material is precipitated in the enlarged interstices, thus rendering growth permanent. This method of growth is most conspicuous, perhaps, in the germinating seed. The rising temperature in spring produces in the seed substances of higher osmotic pressure (with greater attraction for water) than the substances from which they originate. The result is that

water enters the seed; by the pressure of the water within the cells their walls are stretched out and the seed grows. The chemical and osmotic changes are the sources for the energy which is needed to overcome the resistance to growth.

To see whether I could determine what are the mechanical causes of growth in animals, I began at Naples some experiments on Tubularia mesembryanthemum. I chose long stems belonging to the same colony and distributed them in a series of dishes containing sea-water of different concentrations. In some of the dishes the concentration had been raised by adding sodium chloride, and in others it had been lowered by adding distilled water. According to the laws of osmosis the amount of water contained in the cells of these tubularians differed with the concentration of the sea-water, the amount being greatest in the most diluted solution and least in the most concentrated solution. If now in reality the mechanics of

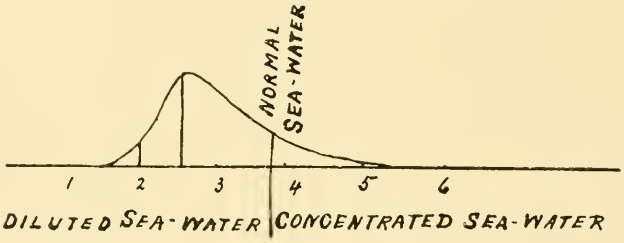


FIG. 13.

growth are the same for animals as for plants, it must result that the more diluted the sea-water the more rapid would be the growth in the tubularian stem. Of course, at last, a limit is reached where the water begins to have a poisonous effect. It was found, indeed, that within certain limits of concentration the increase in the length of the stems during the same period was greatest in the most diluted and least in the most concentrated sea-water. It is remarkable that the maximum of growth took place not in sea-water of normal concentration, but in more diluted sea-water, though that of course may not be the case in all animals. The following curve (Fig. 13) will give an idea of the dependence of growth upon the concentration of the sea-water in Tubularia. The values for the amount

of sodium chloride, in 100 cubic centimetres of sea-water, are represented on the axis of the abscissa, the values for the increase in growth on the ordinate axis.

These and similar experiments, which on account of lack of time I cannot mention here, show that growth in animals is determined by the same mechanical forces that determine growth in plants. An obstacle to such a conclusion seems to lie in the fact that many plant cells have solid walls, while such is not the case in most animal cells. But the solid cell-wall does not determine the peculiar character of growth. This character is determined first, by chemical processes within the cell, which result in a higher osmotic pressure, and, secondly, by the osmotic qualities of the outer layer of protoplasm, which allows water to pass through freely, but does not allow all salts dissolved in it to do the same. Both these qualities are independent of the solid cell-wall, and I see no reason why the animal cell should not agree in these two salient features with the plant cell.

In order that the foregoing explanation of the mechanism of growth in the animal cell might be based only upon known processes, it was necessary to find out whether, indeed, in case of growth, chemical processes of such a character take place that there are formed substances of higher osmotic pressure than those from which they originate. Every one knows that by practice our muscles increase in size. No satisfactory explanation of this fact has been given. If my interpretation of the method of growth was correct, I must expect that during activity there are formed in the muscle substances which determine a higher osmotic pressure than those from which they originate. This is exactly the case. Ranke had already shown that the blood of a tetanized frog loses water and that this water is taken up by the muscles. In experiments which were carried on by Miss E. Cooke in my laboratory, we were able to show directly that during activity the osmotic pressure inside the cell-wall is raised. We determined the concentration of a solution of Na Cl, or rather of a so-called Ringers mixture, in which the gastroconemius of a frog neither lost nor took up water. We found that while this concentration for the resting

gastroconemius was about 0.75 per cent. to 0.85 per cent., for the gastroconemius that had been tetanized from 20 to 40 minutes it varied from 1.2 per cent. to 1.5 per cent.

This increase of osmotic pressure inside the muscle cell leads, during normal activity, to a taking up of water from the blood and lymph, and the consequence is an increase in volume. The same muscle, as soon as it ceases to be exercised, begins to decrease in size. Activity, therefore, plays the same rôle in the growth of a muscle that the heat of spring plays in the growth of the seed.

Upon endeavoring to determine whether the function of segmentation, like other functions of growth, is influenced by the amount of water contained in the cell, I found that by decreasing the amount of water in the ovum of the sea-urchin segmentation is retarded, and that by using a sufficiently high concentration of sea-water it may be stopped entirely. Therefore the amount of water contained in the cell plays still another rôle in the process of organization and influences the process of cell division.

IV. The Artificial Production of Double and Multiple Monstrosities in Sea-Urchins.

The idea that the formation of the vertebrate embryo is a function of growth has been made the basis of the embryological investigations of His. In a masterly way, His has shown how inequality of growth determines the differentiation of organs. In the blastoderm of a chick, for example, the first step in the formation of the embryo is a process of folding. There originates a head fold, a tail fold, a medullary groove and the system of amniotic folds. According to His, all these processes of folding are due simply to inequalities of growth, the centre of the blastoderm growing more rapidly than the periphery. It can be shown, very simply, that such a process of unequal growth must, indeed, lead to the formation of exactly such a system of folds as we find in the blastoderm of a chick. If we take a thin, flat plate of elastic rubber, such as is used for medical purposes, and lay it on a drawing-board, we can

imitate the stronger growth in the centre by sticking two tacks into the middle of the rubber, a short distance apart, and then pulling them in opposite directions. In this way we imitate unequal growth, the centre growing faster than the periphery. If we, then, fix the tacks in the drawing-board, so that the rubber in the middle remains stretched, we get the same system of folds shown by the embryo of a chick. I mention this way of demonstrating the effects of unequal growth as the ideas of His are still doubted by some morphologists.

His raised the question, Why is growth different in different parts of the blastoderm? But instead of trying to answer it from the physiological standpoint he answered it from the anatomical standpoint. According to His, the different regions of the unsegmented ovum correspond already to the different regions of the differentiated embryo. But this so-called theory of preformed germ-regions gives no answer to the question, Why do some parts of the embryo grow faster than others? Nevertheless, it is not necessarily in opposition to the theory of growth that I offered in the preceding chapter. Starting with the idea of His, we may well imagine that the different regions of the ovum are somewhat different chemically, and that these chemical differences of the different germ-regions determine the differences of growth in the blastoderm. Thus the phenomena of heteromorphosis would show that, in some animals at least, the arrangement of preformed germ-regions may be changed by gravitation, light, adhesion, etc.

But, from the standpoint of causal morphology, it must be asked what determines the arrangement of the different germ-regions in the ovum. If we answer "heredity," causal morphology can make no use of such an explanation. Our blood has the temperature of about 37°, but although our parents had the same temperature its heat is not inherited, but is the result of certain chemical processes in our tissues. Still it may be possible that the molecular forces of the chemically different substances of the ovum determine a separation of these substances and at the same time the chief directions of the future embryo.

Driesch has shown[1] that by shaking a sea-urchin's egg in the four-celled stage the four cells may be separated, and each one be capable of giving rise to a complete embryo, which is only different in size from the normal embryo. If the theory of preformed germ-regions with its later modifications were true, we ought to expect that every one of the isolated cells would give rise to one-fourth of an embryo. But it has been said that the artificial isolation of one cleavage cell causes a process of post generation or regeneration. Driesch, moreover, changed the mode of the first cleavage by submitting the ovum to one-sided pressure. In this way the nuclei were brought into somewhat different places from those they would have held in the case of normal segmentation. Still, normal embryos resulted. One might object again that the preformation of the germ-regions existed in the protoplasm, and not in the nucleus. I have made a series of experiments to the results of which these objections cannot be made. I shall describe these experiments somewhat fully, as they have not yet been published, though I cannot enter into details at this place.

I brought eggs of a sea-urchin, within ten to twenty minutes after impregnation, into sea-water that had been diluted by the addition of about 100 per cent. distilled water. In this solution the eggs took up so much water that the membrane (*m*, Fig. 14) burst and part of the protoplasm escaped in the form of a drop (*b*, Fig. 14), which often, however, remained in connection with the protoplasm inside the membrane after the eggs were brought back into normal sea-water. These eggs gave rise to adherent twins, the ejected part of the protoplasm, *b*, as well as the part remaining inside the membrane, developing into a normal and perfectly complete embryo. The part of the protoplasm which at first had connected the two drops, formed the part where the twins remained

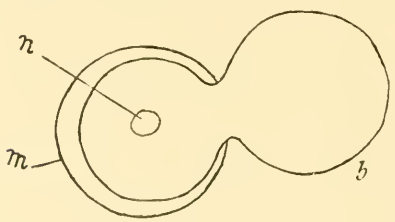


FIG. 14.

[1] *Zeitschrift f. wissensch. Zoologie*, Vol. LIII and LV.

grown together. Of course, it often happened that, by accident or rapid movement, the twins were separated, and they then developed into perfectly normal single embryos. Since we cannot assume that in every case the same part of the protoplasm escapes, we must conclude that every part of the protoplasm may give rise to fully developed embryos without regard to preformed germ-regions. In many eggs a repeated outflow of the protoplasm takes place (Fig. 15). In such cases each of the drops of the protoplasm may give rise to an embryo, and I obtained not only double embryos, but triplets and quadruplets all grown together.

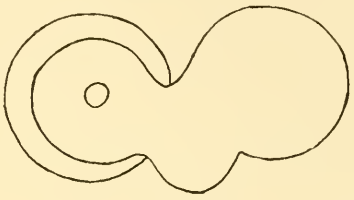


Fig. 15.

In order to understand these experiments more fully, let us follow out the history of development in these double and multiple monstrosities. The ova were put into the diluted sea-water before any segmentation had taken place and while they had still but one nucleus. When parts of the protoplasm flowed out, the nucleus either remained in the protoplasm, inside of the membrane, or passed out with the part that was ejected. Therefore, at first, only one part of the protoplasm contained a nucleus. The other part obtained its nucleus by the cleavage which took place, as follows : In case the nucleus had remained inside the membrane (Fig. 16), the first cleavage took place inside the membrane, the cleavage plane being always at right angles to the common diameter of the two protoplasmic drops (Fig. 16). (Only when the nuclear-spindle had already formed, at the time of the bursting of the membrane, were there exceptions to this rule.) A peculiarity of the first cleavage was that the protoplasm was always divided into a larger sphere (I, Fig. 16), and a smaller sphere (II) which was connected with the extra-ovate.

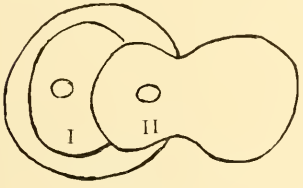


Fig. 16.

The next stage was a normal division of I (Fig. 17), and then II, divided (Fig. 18), the cleavage plane, as a rule, lying

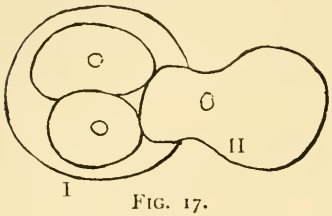


Fig. 17.

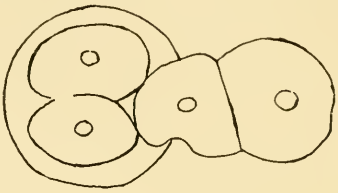


Fig. 18.

in the extra-ovate. In this way the extra-ovate obtained its nucleus, receiving in this case but one-fourth of the whole. It very often happens that the extra-ovate receives its nucleus later, obtaining in that case a still smaller fragment, but, nevertheless, the outcome is a perfectly normal embryo. Later on

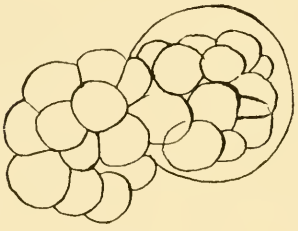


Fig. 19.

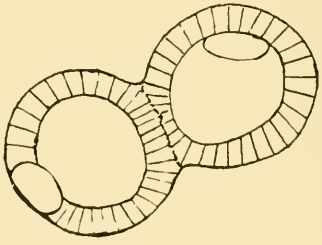


Fig. 20.

the division takes its regular course. Fig. 19 shows the morula stage, and one recognizes already the double monster. Figs. 20, 21 and 22 show double, triple and quadruple gastrulae.

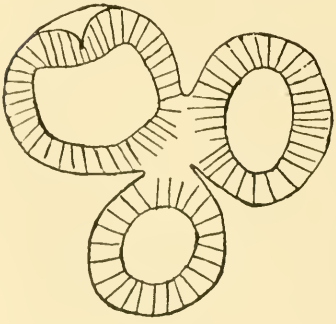


Fig. 21

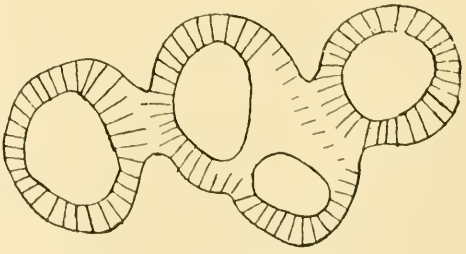


Fig. 22.

Figs. 23 and 24 represent double Plutei and Fig. 25 a triple Pluteus.

It is remarkable that the development of these monstrosities goes on nearly at the same rate as that of the normal embryo, provided they are equally well supplied with oxygen and equally protected from microbes and infusoria. If preformed germ-regions determined the arrangements of organs in the embryo, we ought to expect that these ruptured ova would give rise to single embryos, with a modified arrangement of limbs, and not to several embryos with normally arranged limbs. Nevertheless, it remains true that the development in most eggs takes place in such a regular and typical manner that it seems as if there were a pre-arrangement of some kind. But it is perfectly well possible that this pre-arrangement consists in a separation of different liquid substances in the ovum by the molecular qualities of these liquids. Such a separation, of course, might be called a preformation of germ-regions, but it would be something totally different from what is now understood by that term.

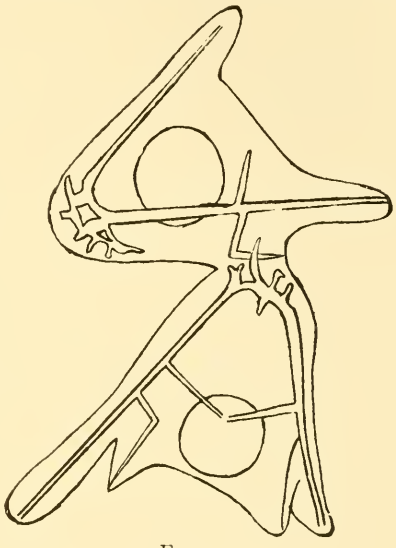


FIG. 23.

V. THEORETICAL REMARKS.

1. All life phenomena are determined by chemical processes. This is equally the case whether we have to do with the contraction of a muscle, with the process of secretion or with the formation of an embryo or a single organ. One of the steps that physiological morphology has to take is to show in every case the connecting link between the chemical processes and the formation of organs. I have tried to show that in a

few cases at least this connecting link was to be sought in the changes of osmotic pressure determined by the chemical changes which take place in the growing organ.

But this fact alone does not explain why it is that we get differences in the forms of organs. In order to understand this we must bear in mind that the processes of growth must necessarily be different for different organs, as for example in the formation of a root, and the formation of a stem. As

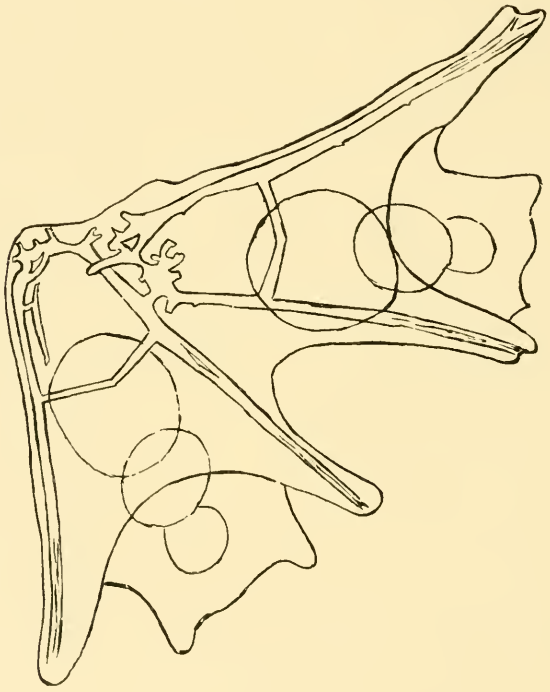


FIG. 24.

growth is a process in which energy is used up in overcoming the resistance to growth, differences of growth can only be determined either by differences in the amount of energy set free in the growing organ or by differences in resistance. Differences in the energy must be the outcome of differences in the chemical processes which determine growth. Therefore we are led to the idea that differences in the forms of different organs must be determined by differences in their chemical

constitution, or, if the chemical constitutions be similar, by differences in resistance to growth. That organs which differ in shape very often are chemically different is a well known fact. The formation of urea in the liver and the synthesis of hippuric acid by the kidneys are the consequences of chemical differences.

In this way we are led through the mechanics of growth to a conclusion which forms the nucleus of Sachs' theory of

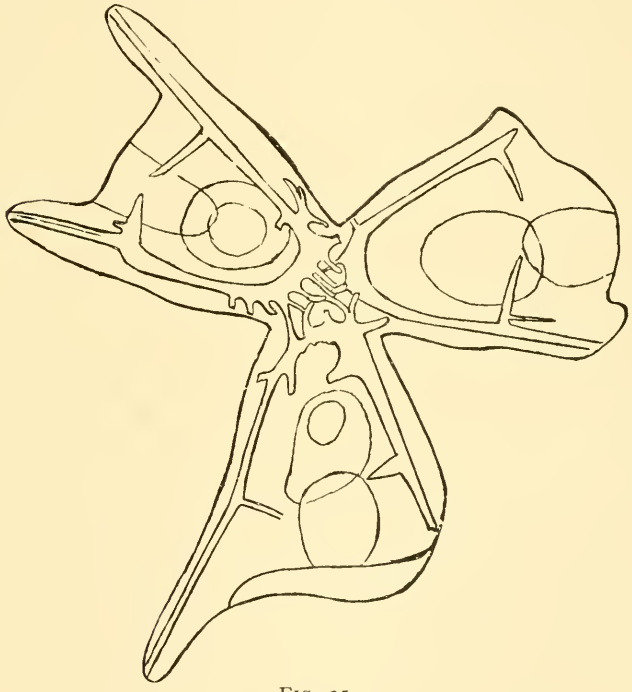


FIG. 25.

organization, namely, "that differences in the form of organs are accompanied by differences in their chemical constitution, and that according to the principles of science we have to derive the former from the latter." According to Sachs there are as many "spezifische Bildungsstoffe" in a plant as there are different organs.[1]

[1] J. Sachs, *Stoff und Form der Pflanzenorgane*, Gesammelte Abhandlungen, Vol. II, 1893.

2. In adopting the theory of Sachs and applying it to animal morphology, we must avoid a mistake very often made even in the case of good theories, namely, the endeavor to explain special cases which are complicated by unknown conditions. Huyghens explained by his theory of light the phenomena of refraction, but he could not and did not attempt to explain the sensations of color. For these phenomena the wave theory of light remains true, but color sensations depend not only on the wave motion of the ether, but also on the peculiar chemical and physical structure of the retina. I think it perfectly safe to say that every animal has specific germ substances, and that the germ substances of different animals differ chemically. Its chemical qualities determine that from a chick's egg only a chick can arise. But it would be a mistake and a falling back into the German Natur-philosophie to attempt at present an explanation of how the unknown chemical nature of the germ determines all the different organs and characters that belong to the species. For instance, the yolk sac of the Fundulus embryo has a tiger-like coloration. We might say that these markings may be due to a certain arrangement of molecules or complexes of molecules (determinants), which later on give rise to the colored places of the yolk sac, but I found that this coloration originates in a manner much more simple. The pigment cells are formed irregularly on the surface of the yolk. The pigment is chemically closely related to haemoglobin, and so its formation may from the first be connected with the formation of the blood corpuscles. But the arrangement of the pigment cells during the first days of development is not such as to produce any definite markings. They lie upon the walls of the blood vessels as well as in the spaces between the capillaries. Later on, however, all of the pigment cells have crept upon the surface of the neighboring blood vessels. I succeeded experimentally in showing it to be probable that some of the substances contained in the blood determine this reaction. These substances, if they diffuse from the blood vessel and touch the chromatophore, make, according to the laws of surface tension, the protoplasm of the chromatophore flow

towards and at last over the blood vessel and form a sheath around it, while the gaps between the blood vessels become empty of chromatophores. In this way the chromatophores are arranged in stripes, and changes in the surface tension, and not a preformed arrangement of the germ, determine the marking. We do not know what processes determine the coloration of animals which owe their markings to interference colors, but the task of deriving such a coloration in the adult from a similar arrangement of molecules in the germ plasm would prove too much even for a genius like Huyghens, and without the possibility of such a derivation the theory is of no use.

3. The reasons why roots grow on the under side of the stem of Antennularia and stems on the upper side, can only be given when the special physical and chemical conditions inside the stem of Antennularia have been worked out. At present we can only think of possibilities. It is possible that the hypothetical root substances of Sachs may have a greater specific gravity than the substances which form stems, and therefore take the lowest position in the cell. Since outgrowth can take place only at the free surface of a stem or branch, roots could grow only at the under side and stems only at the upper side of an element. But there are still other possibilities which we must omit here. In the case of Margelis and other hydroids, it might happen that contact with solid bodies produced an increase of surface in the touched elements in case they contained specific root substances, while the opposite took place in the case of elements containing polyp substances. The consequence would be an increase in the surface of the roots if they came into contact with solid bodies, while polyps only would grow out in the opposite direction. I found, indeed, in some forms at Naples that roots of hydroids which grew free in the water began to grow much faster and to branch off more abundantly when brought into contact with solid bodies. But in these cases we must wait with our attempts at explanation until the physical and chemical conditions for the form are worked out. For the same reasons I will not go into a discussion of the question of what determines the polarization of

animals like Cerianthus. It may suffice to suggest the possibility that in polarized animals the tissues or cells may have such a peculiar structure as to allow the specific formative substances to migrate or arrange themselves only in one direction, while in cases of heteromorphosis migration or arrangement in every or in several directions is possible.

4. The ovum of a sea-urchin under normal conditions gives rise to but one embryo. This circumstance is due simply to the geometrical shape of the protoplasm, which, under normal conditions, is that of a sphere. When we make the eggs burst, the protoplasm outside the egg membrane and that which remains within it assume spherical forms, by reason of the surface tension of the protoplasm. When this happens, as a rule, we get twins, if two separate segmentation cavities are formed, and only one embryo, if both cavities communicate with one another. Whether the first or the second case will happen depends upon the molecular condition of the part of the protoplasm connecting the two drops. Therefore, the number of embryos which come from one ovum is not determined by the preformation of germ regions in the protoplasm, or nucleus, but by the geometrical shape of the ovum and the molecular condition of the protoplasm, in so far as these circumstances determine the number of blastulae. In my experiments, I got double or triple embryos when the ovum formed a double or triple sphere, as every sphere determines a blastula. In Driesch's experiments, one single cell of the four-cell stage necessarily formed a whole embryo after it had been isolated, as it assumed the shape of a single sphere or ellipsoid. Of course, there must be a limit to the number of embryos that can arise from one egg; but the limit is not due to any preformation, but to other circumstances, the chief one being that with too small an amount of protoplasm the formation of a blastula — from merely geometrical reasons, as there must be a minimum size for the cleavage cells, — becomes impossible. Without the formation of the blastula, of course it is not possible to get the later stages which are determined by the blastula.

5. The formation of the embryo begins with the formation of the gastrula. The hollow sphere (the blastula, Fig. 26) begins to fold at one place (*a*, *c*, *b*, Fig. 26), and an invagination takes place (Fig. 27). The formation of the fold (*a*, *c*, *b*, Fig. 27) is due, undoubtedly, to unequal growth. The fact that invagination, and not evagination (Fig. 28), takes place

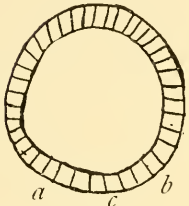


FIG. 26.

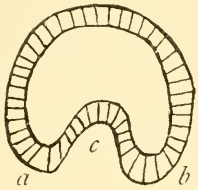


FIG. 27.

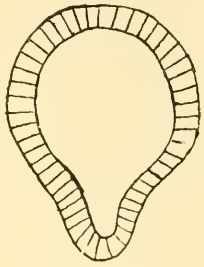


FIG. 28.

depends upon other conditions. So far as the first phenomenon is concerned, there are different possibilities by which unequal growth may lead to a process of invagination. It is possible, for instance, that the part *c* (Fig. 26), between *a* and *b*, may begin to grow more rapidly than the rest. But such an unequal growth can only be the outcome of a chemical difference between the parts *a*, *c*, *b*, and the rest. Such a distribution of chemically different material exists in the blastula which originates from the extra ovate, as well as in the one which develops within the membrane. In the normal ovum this distribution of material finds, perhaps, its first morphological expression in the formation of micromeres. But Driesch has shown that all possible variations in the formation of micromeres may occur without affecting the formation of the embryo. A necessary condition for the formation of the embryo is the chemical differentiation which leads to unequal growth; but whether this differentiation exists from the first, or whether it takes place in the eight or the sixteen or the sixty-four-cell stage is of minor importance. In order to demonstrate this I made eggs burst in the two, four, sixteen, and sixty-cell stages. In all cases except the sixty-cell stage I produced the doublets, triplets, etc. The only condition

necessary was that one part of the protoplasm should flow out. The chief facts for causal morphology are the chemical differences and the local distribution of the chemically different material by molecular forces.

In regard to the question why under normal conditions the entoderm is pushed inside the segmentation-cavity I wish to direct attention to a point that, as far as I know, has not yet been considered. The sea-urchin's egg, during the first stages of development, has a higher specific gravity than the sea-water. In the blastula stage, however, its specific gravity decreases and the egg begins to float and comes at last to the surface of the water. This is possible only through the eggs taking up substances which have a lower specific gravity than the sea-water, and the only substance that could, in this case, be taken up is water. As the cells themselves do not become larger, we must assume that by a process of secretion the segmentation-cavity is filled with a liquid of lower specific gravity than sea-water. In this way the cells of the blastula come to have their peripheral surfaces in contact with sea-water while their central surfaces are in contact with a fluid containing less salts. This might result in such differences of tension, between the inner and outer walls of the blastula as to determine invagination. I found, last year, that when I raised the eggs in diluted sea-water, evagination (Fig. 28), instead of invagination, took place. Herbst got the same result by using solutions of lithium salts.[1] It is possible, too, that in these cases the hydrostatic pressure within the segmentation-cavity is increased, and this increase of pressure may have a direct or an indirect effect upon growth.

It is for further investigation, and not for speculation, to settle all these questions in detail. I only wish to point out where, and how far, there exists a relation between substance and form in the early stages of the development of the sea-urchin.

I have chosen the name Physiological Morphology for these investigations, inasmuch as their object has been to derive the

[1] Herbst, *Zeitschr. f. wissensch. Zool.*, Bd. 55.

laws of organization from the common source of all life phenomena, *i. e.*, the chemical activity of the cell. In what way this is to be done is indicated in the chapter on the mechanics of growth.

But the aim of Physiological Morphology is not alone an analytical one. It has another and higher aim, which is synthetical or constructive, that is, to form new combinations from the elements of living nature, just as the physicist and chemist form new combinations from the elements of non-living nature.

FOURTH LECTURE.

DYNAMICS IN EVOLUTION.

JOHN A. RYDER.

The statement that energy, in its kinetic and static forms, has been a factor in the production of the shapes of organisms, does not admit of question at the present time. In order that the data in support of this statement may be presented in their simplest forms, it is necessary to begin with the consideration of the physical causes of the configuration of some of the simplest unicellular organisms. In this way it will eventually be possible to pass from the consideration of the unicellular to that of the multicellular type, viewed from the same standpoint. It requires but little familiarity with the principles of physical science to discover that similar laws hold with respect to the behavior of fluid and semi-fluid bodies, notwithstanding the fact that we may have before us, in one case, a so-called "dead" fluid body, and in the other, a fluid or *fluent* "living" chemical compound or mixture of compounds. Though the one presents the phenomena of 'dead,' and the other of living, matter, both exhibit the similar and common properties of fluids, namely, those of surface-tension, viscosity and adhesion to the surfaces of other bodies, with which they are brought into contact. Under certain conditions, also, the semi-fluid cells of living bodies undergo reciprocal deformation or alteration in configuration, as a result of the interaction of the inherent forces above specified, in ways so precisely similar to those seen when a number of semi-fluid, or fluid dead masses, are juxtaposed or brought into contact, that the resemblances are seen to be due to the coöperation of closely similar, if not identical, forces and properties.

This remarkable resemblance of the physical behavior of semi-fluid living protoplasm to that of semi-fluid dead matter of certain kinds, such as the oils, has led me to apply the term *cytophysics* to the study of the physical properties of the substance of the cell. The convenient terms *cytostatics* and *cytokinetics* very naturally follow from a consideration of the contrasted states of rest and activity presented by the "living" cellular unit of organization. The cytostatic state will be one in which no visible physical activity, other than its secular metabolism, will characterize the cell, during which time, also, the parts of its substance will be in a condition of statical equilibrium in respect to each other, and the cell will, so long as this equilibrium lasts, maintain a constant configuration. The cytokinetic state, on the other hand, is one in which the visible or invisible parts of the cell are undergoing a displacement in respect to each other, as a consequence of which the cell as a whole will manifest changes of configuration.

Free and interfacial surface-tension is probably the most important factor in determining the shapes of cells, since there is associated with it, in multicellular organisms, a complicated series of definite and constant space relations and reciprocal interactions, as respects the cells of definite tissue-tracts, that grow out of the very conditions of combination of those tracts. Still more important, perhaps, are the incessant unequal disturbances of surface-tension, due to metabolic changes, at different points on the surfaces of cellular masses of plasma — a fact beautifully illustrated by the proteus animalcule, *Amœba proteus*. Differences of surface-tension are thus developed at different points and times coexistently and consecutively which lead to the assumption of the most diverse and inconstant shapes by the bodies of these lowly organisms. Such differences of surface-tension are, however, themselves caused by chemical and molecular transformations at definite, but previously unspecifiable, points at or near the surface of the mass of plasma. These processes are, therefore, ultimately to be associated with molecular or chemical transformations, the production of heat, the disintegration and integration of living matter.

But, it will be asked, what is surface-tension? If a drop of water falls through a vacuum, as long as it is falling in space it will present very nearly the form of a sphere. This is due to the fact that the very outermost layers of molecules which lie at insensible distances apart, attract each other with a force that is apparently very much greater than that developed by the action of gravitation. It results from this that the remaining molecules contained within this outer molecular film are held as within an elastic bag, the walls of which are of exactly the same strength at every point. This exactly equal strength and elasticity of the outer molecular film at every point on the surface also causes it to fall into a condition of spherical, statical equilibrium. This is true because the molecules at every point on the surface of the drop are of exactly the same size, therefore every one attracts its fellows that are in contact with it, with exactly the same force at every point on that surface. Now, let any change in the dimensions of these molecules occur at any point on the surface of such a fluid sphere, such as may be accomplished by chemical transformations and recombinations, such as oxidation or decomposition, and it must follow that the surface-tension or reciprocal pull of the molecules upon each other, adjacent to the point of such disturbance, must be increased or diminished. The inevitable result of this will be that the form of the drop must instantly change in order that a new condition of statical equilibrium may be attained. The surface of the drop is finite and returns in every direction upon itself since it is approximately spherical. Any deformation of the drop due to surface-tensional disturbances will therefore affect the shape and curvature of some or all of the surface of the drop so that its shape may become very irregular, provided its surface-tension be disturbed at a number of irregularly distributed points simultaneously; but, since the drop is a finite mass made up of solid particles moving freely among themselves, no matter how much the drop may be deformed or how irregular it may become, its most superficial layer of molecules will always form a closed surface. This fact is important, since, no matter how irregular in shape an *Amœba* may become, its outermost stratum

of molecules always form a closed surface and a molecular envelope for the organism.

Since the molecules within a semi-fluid mass also move freely over one another there is friction developed between them. This friction is known as *viscosity*, and differs in fluids of differing densities and chemical compositions, just as, in fact, the surface-tension of a unit-surface, formed of different fluids, differs, owing to the differing dimensions and properties of the superficial layer of molecules of each fluid. The specific viscosities and surface-tensions manifested by any given fluid are therefore correlated, so that we may infer that these two properties of fluent living matter are also correlated. Since, again, living matter, as found in the cell, is not a homogeneous body or compound, it will be plain that the correlative disturbances in viscosity and surface-tension, due to the processes of metabolism and the associated interplay of osmotic changes, are so complex, when considered together with still other facts, that it will be difficult, if not impossible, in the present state of our knowledge, to trace *all* the steps of their interrelations and interdependences in any given case.

The recent progress in the study of the process of fertilization or conjugation shows that dynamical considerations must here also be taken into account. As is well known, the nucleus and archoplasm undergo alternate expansion and contraction in linear dimensions. The male and female pronuclei increase greatly in size during the phases just before conjugation. In the egg of *Ascaris*, for example, I have noticed that the pronuclei soon assume a globular form and rapidly grow into large spherical bodies by absorbing substance from the surrounding cytoplasm. This rapid growth and spherical form show that surface-tension is being disturbed uniformly over the whole surface of the nucleus, otherwise *its form could not remain spherical*. The remarkable growth of the rays of the asters has the same meaning and must also be interpreted, in part, as a physical process involving radial interdiffusion of heterogeneous molecules to and from the centrosomes into the nucleus and cytoplasm, consequent osmotic disturbances, metabolism and changes of surface-tension. The comparison of the astral

figures, with their rays extending in every direction from the archoplasm, with the rays developed on the surface of a fluid when another fluid of definite properties is dropped upon the former, leading instantly to the production of a "cohesion figure" with rays extending out in every direction, is no mere analogy. In the case of the archoplasm, the rays with their lines of microsomes are probably the effects of diffusion of one kind of plasma, the archoplasm, through another, the cytoplasm, and is therefore not necessarily a phenomenon of contractility, but simply an interdiffusion of the unlike substances produced by the metabolism of growth, which tends to reëstablish statical equilibrium among the parts of the molecular system represented by the cell. This diffusion or osmotic redistribution is conditioned at every step by surface-tension in precisely the same way that the rays of many Heliozoa and Radiolarians are conditioned in water by states of unequal surface-tension at very close but nearly equal intervals over a spherical surface, so that a summation of these uniformly distributed and seemingly conflicting surface tensional forces does not interfere with the maintenance of the spherical figure by the body of the organism. The alternate inflation and collapse of the nucleus during fertilization, and growth during indirect cell-division, is a rhythmical process, and we may characterize it as the *diastole* and *systole* of the nucleus. The alternate extension and retraction of the rays of the archoplasm, as I have observed in the egg of *Ascaris*, is similarly an osmotic or metabolic diastole and systole of the radial figures formed by it, which is intimately associated with and absolutely conditioned by metabolism and osmosis, as the direct experimental researches of Prof. Jacques Loeb have rendered exceedingly probable. Unless the processes of karyokinesis are traced with the utmost caution in the light of dynamical and physiological considerations, there is great danger of our misinterpreting the facts and of assuming that certain of the phenomena guide and control others. It may indeed be possible that embryologists have been until now steadily confounding ontogenetic effects due to the physical processes of growth, as visualized in karyokinesis, with their causes. I anticipate that this

remark will meet with a decided denial from most morphologists ; nevertheless it seems opportune to warn them that the problems they have before them can never be settled by purely morphological methods. If my contention that all ontogenetic problems must be approached by a most intimate combination of the methods of physical, physiological and morphological research is true, we are still far from having anywhere an ideal biological investigator. If it is true that we have been mistaking ontogenetic effects for ontogenetic causes, what a mass of speculation must probably be set aside. All that will remain, in fact, will be the many most valuable and beautiful results of observation made by our foremost morphologists.

It is not intended to thus minimize in any way the great and increasing value of morphological research ; what is really meant is, that there is danger of overrating the importance of morphology to the injury or exclusion of other disciplines of paramount importance. If, as we must suppose, there is such a perpetual flux and interflux of particles and molecules going on throughout the plasma of an egg, owing to the metabolism due to respiration and the consequent surface-tensional and osmotic disturbances during the earliest steps of development, it becomes inconceivable that any such morphologically conceived and entirely hypothetical bodies as "ids," "idants," "determinates," etc., can have a stable existence. The existence of such bodies as fixed entities of finite complexity is absolutely disproved by experiments of the most varied character in separating the first two or four blastomeres of the egg, since it is then found that, in each of these blastomeres, there still inheres the power to produce a perfect embryo. The hypothetical *ids*, *determinants*, *biophores*, *gemmules*, etc., must, therefore, be supposed to be capable of being halved and then quartered without destroying their potentialities. These and numerous other difficulties that cannot be discussed here, render it exceedingly probable that we must look in altogether another direction for an explanation of the processes of ontogeny, viz., to a study of the modes and conditions of the manifestations of the energies that constitute the "life" of the simplest organized forms.

In the very simplest unicellular organisms we have also unicellular molecular mechanisms of a most peculiar kind. The fearfully complex molecular structure of these mechanisms conditions their actions, their forms and powers, no less than does the nature of the watery media, in which these first-lings of life, as well as the germs of higher organisms, must in common develop. In the belief that the study of the "living" mechanism of some of the lowest types of organized existence, in relation to and as affected by their not-living surroundings, might throw some light upon the origin of their forms, the writer has here, in the main, taken up the problems thus raised as physical ones. In the belief, also, that these studies have not been entirely fruitless, the following evidence is offered.

An *Amœba proteus* may be compared to a smoke- or vortex-ring of particles that has been greatly modified, owing to the very complex interaction of disturbances of the surface-tension of its outer enveloping film of molecules, the viscosity of its own plasma, its gravity and power of adhesion to other bodies. If we conceive a smoke-ring to have become a viscous semi-fluid body with an outer film of molecules, and that this ring has contracted until the central opening in it has completely closed, we shall have a mechanism which may be compared in detail with an Amœba in motion, provided only that we modify it still further and in such ways as we are obliged to suppose that the combination of the four forces above specified coöperate in order to produce and maintain the form of an amœboid organism. Verworn[1] has already referred to the flux of the particles of the Amœba through itself, in discussing the general subject of contraction, but as he has not understood the complexity of the process, we need not here concern ourselves further than to say that he has failed to correctly interpret this vortical flux of amœboid organisms. There is, however, such a central flux of particles through the centre of the body of an amœboid, as any one can soon convince himself by carefully observing the behavior of a large living proteus animalcule. The chemical transformations that go on

[1] Bewegung der lebendigen Substanz. Jena, 1892.

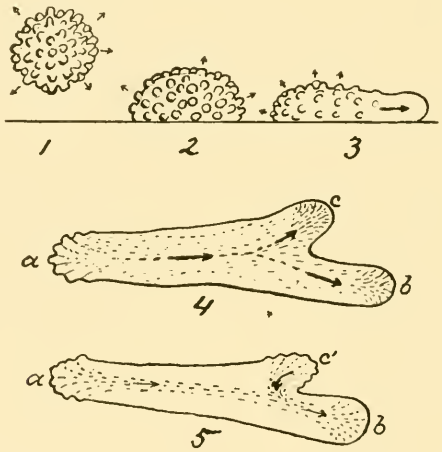
within the body of an amœboid, amongst its molecules, are responsible for disturbances of the surface-tension at one or more points over its surface. It is certain that the chemotropism, or affinity for oxygen of its surface molecules, as Verworn holds, is essential to this; but we must remember that in order to call forth local disturbances of surface-tension in this way, there must also be a locally exaggerated chemotropism, or affinity for oxygen, at some one point on the surface of the organism. This point has apparently not been made clear by Verworn, but it is essential to a clear understanding of the processes presently to be described. How the positions of such local disturbances of the chemical complexity of the surface-layer of molecules of an amœboid are determined, we do not know. That they are definitely determined, according to some definite law, we may be certain.

As every one knows who has ever watched a proteus animalcule under the microscope, the "anterior" pole of the creature, for the time being, is tensely filled with its own substance, so as to present a rounded, full "anterior" extremity while in motion. "Posteriorly," on the other hand, the creature is found to present a wrinkled, partially collapsed appearance. A study of the motions of the organism discloses the fact that its substance is flowing through itself. The central part of this current is moving most rapidly along the middle of the body, while toward the sides it is observed to become progressively slower until the outer layers of its substance, along the sides of the creature, are seen to come to rest. It thus results that the outer layers of molecules, along the sides, form a sort of shell or tunnel through which the central current flows. This flux, however, means that there must be a continual or fitful rupture of the "anterior" end of the organism in order to let some of itself escape out of itself in front, in order to let some of itself flow into itself behind, in order that some of itself may thus continuously flow through itself in order to make the progressive forward motion of itself possible. In this way the substance of the "posterior" part of the organism can be picked up and carried through the tunnel-like physical shell, formed of the outer layers of molecules of the organism,

and thus be transported forwards and poured out "anteriorly." It will readily be seen that, in order to do this, work must be performed, energy dissipated. This energy is dissipated in reëstablishing a dynamical balance between the parts of the dynamical system represented by the molecular aggregate that we behold in the body of the *Amœba*. We have here before us a finite molecular mechanism, or dynamical system, every part of the surface of which returns upon itself. Any disturbance of the equilibrium of the molecules at the surface of that system will provoke a deformation of the whole. If the disturbance is great enough at any one point, there will inevitably be developed a vortex-ring motion of the particles amongst themselves, due to the friction of the constituent molecules. If this vortical motion of the particles of an amœboid mass of "living" matter be due to a preponderating superficial disturbance of the equilibrium of the system at one point on the surface, that alone will suffice to determine the direction of the motion of the whole. For this reason the proteus animalcule has no fixed "head" or "tail" end. "Head" and "tail" seems to be entirely a matter of the combination of inner and outer conditions that determine the point on the surface of the organism, at which the maximum chemical and physical disturbance of surface-tension will occur. These are absolutely determined by the physical processes of the readjustment of the equilibrium of its molecules, in respect to outer conditions, during every consecutive moment of its motions. It is possible, in fact, to show that every change of the shape of this interesting organism is the result of energies inter-acting in a very complex way.

If an Amœba is allowed to fall through the water, its surface-tensions are apparently disturbed with great uniformity over its whole surface, and at nearly equal distances apart. It results from this that short, blunt pseudopodia are pushed out in every direction, as in Fig. 1 and it becomes nearly globular in outline. The moment, however, that the organism touches a plane surface, as in Fig. 2, it flattens out into the form of a biscuit-shaped mass. It now behaves like a mass of dough, and falls into a new condition of

equilibrium, due to its own gravity, its viscosity, and the force with which the particles on its under side tend to adhere to the fixed solid surface upon which it rests. The upper part of the mass now falls into an equilibrium determined by the surface-tension of its superficial molecules and the viscosity or reciprocal friction of the latter upon each other. Gravity, viscosity, surface-tension, and adhesion are the four coöperating agents that now determine its figure. So long as it was floating or suspended in the water, the influence of gravity and adhesion operating in relation to a fixed surface were excluded. We thus see how completely this organism is the creature or subject of energy-conditions in this state.



In the next phase of its motions this is still further illustrated. In Figs. 1 and 2, short, blunt pseudopodia are being protruded in all directions; but in Fig. 3, the sequence of events changes, and the organism begins to take up its march by means of a vortical flux of its substance across the surface upon which it rests, and in the direction of the arrow. Surface-tensional disturbances of greater magnitude have evidently affected the right side of the mass, and one or more of the small original blunt pseudopodia at this point have been merged into a strong, single "anterior," pseudopodal current, in which a maximum flux of molecules is taking place in the

direction of the motion of the whole organism. This maximum vortical flux of the material particles of the amœboid in this new direction will result in elongating the organism in th direction of the motion thus set up. The elongation of the organism is, therefore, due to the flow of its own particles amongst and through themselves. We are thus made aware of the fact that this simplest organism is elongated in the direction of its own motions, as a consequence of the continuous readjustment of the internal equilibrium of its parts in respect to influences affecting it from without. The shape of this organism, at every instant of its motions, is, therefore, mechanically caused.

We may pursue our analyses still further. If we now look down upon such an amœboid organism from above, when moving upon a plane surface, as in Fig. 4, we find that it is not only elongated in the direction of its motion, but that its anterior end is tensely filled with substance; its outer layer of molecules at the "anterior" end is tensely stretched. At the tail end the organism is wrinkled or papillated; this wrinkled or papillated appearance of the posterior end of the organism is due to physical causes, as the following observation proves: In Fig. 4 there are two anterior pseudopodia, — *b* and *c*, — through which there are fluxes of particles in progress at about the same rate. The vortical current is seen to divide and to flow into both these pseudopodia, thus keeping the outer molecular film over both tense and rounded. Let, however, a disturbance of the surface-tension occur at the end of *b*, while at the end of *c'* this disturbance subsides, as in Fig. 5 and there will be a flux of particles out of *c'* — in the direction of the arrow — into *b*, and *c'* will lose its tense, full outline, and its surface will become wrinkled or papillated, just as appears at the "tail" end of the organism at *a*. In every detail of the morphology of this organism, we therefore discover that physical or "physiological" agencies are operative in determining its figure. We are, therefore, in a position to affirm, with positive certainty, that the morphology of this organism has little significance until its *actions* and their physical causes have been studied. This is only one of

many reasons why the present speaker holds that all purely morphological work is one-sided, and incapable of dealing with the deeper problems of biology. In the same way, physiology alone is equally incapable of dealing with the great general problems in biology. Neither of the two disciplines *alone* can ever, by any stretch of imagination, command the power to produce *a theory of life*. Such an expectation is simply fatuous.

The many other dynamical phenomena, especially the singular manner in which the nucleus of an *Amœba proteus* is caused to oscillate back and forth within its body within certain limits, all take place in accord with the views above developed. For example, the curious lateral flow of the plasma of *Amœba proteus* when in a fully extended condition, along the sides of the body on either side of its posterior and middle thirds, may be explained by the physical tendency of this part of the organism to adhere to the substratum upon which it is moving. This lateral spreading does not take place at its "anterior" end because here the influx of molecules from behind is taking place so rapidly that this spreading due to adhesion has not yet had time to take place. This spreading due to adhesion causes the central current to be narrowed and elevated so that the discoidal nucleus is lifted and rolled along on its edge in this narrow elevated part of the body of the organism as if passing through a tunnel with its sides wider than its floor. Rolled along in this way through this narrow passage by the vortical current of plasma, the nucleus is finally arrested by contact of its edge with the molecular boundary wall of the organism, and is thus automatically kept from being bodily rolled to the outside of the plasma of the organism. Reaching a certain point at the "anterior" end of the mass of plasma, it also falls over on its side, wedged between the roof and floor of the mass of plasma of which it forms a part. It is thus prevented from passing out of the organism with the vortical current that must now flow past it and on forward to continually form anew the "anterior" end of this singular being that is thus continually turning itself inside outward in front and outside inward behind.

The manner in which the successive rents in the outer molecular skin of the organism are produced is such that a sudden rupture occurs through which a fresh outpour of substance happens over which a new molecular skin is instantly formed continuous with the ruptured edge of the old. These rents do not take place in relatively the same place in succession. For example, rents may occur in the outer film one after the other at points a little off of the extreme central point of the "anterior" end, and alternately a little to the right and left side of the latter. The vortical flux is thus thrown slightly aside alternately to the right and then to the left, then to the right, then to the left again, and so on, indefinitely. It thus results, however, that the summation of these alternating outbursts of substance become the components of the aggregate motion of the whole organism in one general direction.

If a rent occurs at one side of the organism, due to a disturbance of surface-tension at an anterior lateral point, a lateral flux of particles may thus be set up, as a result of which the entire contents of the organism may be sucked up "anteriorly" and "posteriorly" to the point where the new outflow has taken place. In other words, the "head" and "tail" ends of the organism may be thus caused to flow in opposite directions into the lateral outburst and the whole organism take a new direction of motion with all its parts oriented in respect thereto. *Amœba proteus*, therefore, cannot move except by developing a vortical flux of particles, and it therefore is a "living" vortex-ring of particles. These outbursts, in large individuals, occur successively at short intervals of time, so that the motion of the creature is fitful. In young proteus animalcules the bursting out or pouring forth of the plasma at the "anterior" end may be continuous, so that the motion is uniform and an ideally perfect type of the "living" vortex-ring of particles is realized.

Every pseudopodium is, however, so long as it is being protruded, a vortex-current in which the motion of the particles is swiftest at its center and at its tip, while at the sides the particles are quiescent and form a shell through which the central ones are moving. The pseudopodia, therefore, divide

the vortex-current of particles into diverging currents, the summation of the motions of which may, when the organism is moving on a plane surface, become resolved mechanically into a single motion of translation in one direction.

When the organism is freely suspended in water it protrudes pseudopodia equally in every direction and assumes a globular figure, somewhat like a Heliozoan or Radiolarian, because its surface-tension is now being disturbed at nearly equally separated points over its whole surface. It therefore falls into a condition of spherical equilibrium, since the components of all the surface-tensions are resolved at the centre of the organism. The moment it touches a fixed surface, distortion of the formerly globular organism takes place under the influence of gravity and adhesion, and the vortical flux of particles that is now set up is different in nature from that which took place in every direction when it was in the suspended globular condition. There is at once a maximum flux of particles in the direction of the point at which the most active disturbance of surface-tension occurred, and the resulting current becomes very massive so that the whole contents of the organism, except the nucleus and water vacuole, flow forward as a massive pseudopod, that is somewhat flattened or depressed by the action of gravity and adhesion, but which is much larger than any of those produced while in the spherical condition. How little Verworn has appreciated and understood these complex processes may be judged by any one who has read his work on the motion of living substance. He has affirmed what does not exist in regard to small *Amœba proteus*, where the flux is continuous and where no retreat of particles to the nucleus, such as he postulates, can take place for hours together. In short, Verworn has totally failed to understand the real significance of these complex phenomena. Greef, also, has made assumptions with regard to amœboid motion, especially the existence of muscular fibrils, which by no stretch of the imagination can be conceived to hold of the proteus animalcule.

Verworn has also failed to understand the physical reasons why small blebs or pseudopodial warts were produced along the sides of a retracting pseudopod. He speaks of "stimulation"

(*Reiz*), a word which to me has a very indefinite meaning. The production of these blebs or incipient pseudopodia, which I have often observed, and in a great variety of forms, may be equally well explained by surface-tensional disturbance, the viscosity of the plasma, etc., and not necessarily as caused by a retreat of the substance toward the nucleus, as claimed by Verworn, in order to receive a reinforcement or accession of new nuclear molecules.

The differentiation of the substance of an amœboid, such as *A. proteus*, into an outer hyaline layer and a central or medullary part, — the so-called "endosarc" and "ectosarc," — is again a purely physical phenomenon. The surface-tensional forces *must* be exerted, for physical reasons, between molecules of approximately the same dimensions, otherwise there could be no tolerably coherent and stable outer stratum formed. In other words, the molecules of the outer layers are reciprocally attracted to each other with a greater force at insensible distances apart, than the larger and coarser particles are attracted by the small uniform molecules at the same intervals apart. The materials of an amœboid are thus dynamically sorted into two kinds, in virtue of their differences of attraction for one another. It thus comes about that "endosarc" and "ectosarc" are merely names for the results of a dynamical process that, so to speak, sorts the particles of a living amœboid into two kinds. The small surface-particles or molecules necessarily attract each other under such conditions of dynamical advantage over and above their attraction for the larger particles, that the latter are constantly driven inward and kept there for this reason.[1] In this case, again, a morphological fact is only understood when subjected to dynamical analysis.

In this way one might go on and subject every detail of the organization of an amœboid to physical scrutiny, and show that complex energy-combinations determined every detail of its structure. Not a single amœboid form that I have yet encountered has failed to disclose this mechanical and dynamical complex of agencies as factors competent to

[1] A geometrical diagram would be needed to make this statement perfectly clear.

determine its figure at every instant of its existence. There may be those, however, who will find fault with my identification of the motions of an amœboid with that of a whirl- or vortex-ring of particles. It may be well, also, to explain here that this notion has nothing to do with the physical conception of Lord Kelvin respecting the existence of vortex atoms. The identification of amœboid motion with a vortex-ring of particles is perfect, provided certain reservations are made that grow out of the very nature of the conditions and nature of amœboid motion. In a typical smoke- or vortex-ring the impulse of rotation that impels all its particles is imparted from without; in the living vortex-ring secular changes in the nature of its constituent particles condition its motions and provoke them. And, while it is true that the energy that drives the living vortex-ring is primarily derived from without, it is not set free until the constituent particles have changed their dimensions, affinities, and reciprocal attractions within the ring, due to causes acting antecedently from without. The living vortex-ring is a constantly changing, closed, and regenerative dynamical system; the other is a dynamical system that derives all its energy from without in the form of a single impulse, and comes to rest after a time, owing to the friction of its superficial particles with their surroundings.

It may be objected, also, that there is only a remote resemblance of a smoke-ring to that of the flux of living particles through an amœboid organism. This objection may be met by the statement, that not only do I suppose the living vortex-ring to be flattened by its gravity, but I also suppose it to have no central opening, and that the median longitudinal stream of particles represents the point where the opening would be in a smoke-ring — now a line in Amœba — along which the central "living" vortex current is flowing. I moreover suppose that the ring is greatly elongated, owing to the viscosity of its substance and the adhesion of the surface of the living amœboid to the substratum upon which it moves. I would not have any one suppose that I imagined that amœboids ever existed that had a central opening in them

like a smoke-ring. I have only used this comparison because of the obvious identity of the living and dead vortices, when proper allowance is made for the physical conditions under which both subsist on account of the different nature of their constituent substances. The outer molecular shell, for example, of a living vortex-ring is fixed to its substratum, and is only involuted "posteriorly," and evoluted "anteriorly" as it moves over a fixed surface. These ideas once clearly grasped, will enable any one to see that, underlying the apparent unlikeness of the smoke-ring to a living amœboid vortex, there is in reality a fundamental similarity. The kind of "contractility" which is exhibited by an amœboid is thus also seen to be fundamentally different in nature from that presented by a muscle, in which contraction is conditioned by a vastly more complex structure.

When an Amœba passes into the resting stage its plasma is very apt to assume an almost perfectly globular form. Its pseudopodia are retracted; its surface becomes smooth, and the whole organism passes into the almost homogeneous, quiescent or lethargic condition of a ball of protoplasm that no longer manifests its characteristic types of motion and irritability, at least externally. Unequal surface-tensional disturbances no longer affect it; it is now under the domination of the same physical influences that determine the globular form of a sphere of oil in a mixture of alcohol and water of the same specific gravity as itself. Its cytoplasmic substance is nearly or quite homogeneous, and the excessively slow and torpid metabolic processes, within the protoplasmic mass of the Amœba, now secrete or pour out a cuticle or envelope over its surface and in which the organism is said to be "encysted." All of these processes are purely dynamical, just as were all of those associated with the motion of the organism. Not only are all of these processes dynamical, but all are also directly "adaptive," in virtue of the fact that the equilibria successively attained are merely a quantitative dynamical response from within a "living" molecular mechanism to a change in external energy-conditions. All "adaptation" is to be so interpreted, so that "natural selection" may be at last resolved into pure energy-factors, and thus brought into coördination with the

rest of the forces of the cosmical universe. The mischievous metaphor, "natural selection," has blinded many naturalists to the real meaning of this expression.

In the same way we may discuss the characters of other amœboids. Each is found to have its own kind of plasma, that behaves differently in each species. Some move more rapidly than others, some have blunt, others long, slender or attenuated pseudopodia ; some may have bent pseudopodia, or have the axes of their straight pseudopodia normal to the surface of the spherical body of the organism ; others obey a different rule in this respect. In some the plasma is clear, in others more opaque ; in some the nucleus is globular, in others flattened. Thus one might go on and show that the different proportions and behaviors of the same parts, in different species, was evidence that totally different molecular mechanisms were simply making their necessarily different responses to the same environment, because of differences in the physical and chemical properties of their constituent plasma. Herein, probably, also lies the whole secret of the difference of power presented by the germs of different creatures. They develop as they do in the case of each species, because of their specific chemical constitutions, and not because there are a lot of "biophores," "gemmules," etc., "superintending" the business of development in a particular way during the evolution of the egg of each form. The preposterous assumption that enough energy can be squeezed into an egg potentially to carry the materials into place that are assimilated during development in order to build an elephant, for example, is worthy of mediæval philosophers, but not of those of the close of the nineteenth century. Every form of energy, including that of "life," is correlated, and is amenable to the same laws. Full knowledge of the mode of operation of the material energy-complexes that we call "living," will disclose the true theory of morphology and the true meaning of life as well.

These, here incompletely reported, observations make it tolerably clear that amœboid motion is worth studying, in order to get clear notions of how living motions and energies are operative in one of the simplest organisms known to the zoölogist.

They also make it very evident that observers have hitherto allowed purely morphological considerations to becloud their vision. In order to discover the meaning of the morphology of the simplest organisms, their actions must be subjected to the most searching analysis. These observations have proved that the functions of an organism are also functions of its form. It has been pretty clearly established, by what has been said above, that every action of the Amœba was followed by a morphological change. In the belief that this same thing holds good throughout the whole animal and vegetable world under most complexly interacting energy-conditions, I will predict what I believe will yet be possible, viz., the discovery of the true causes, in detail, of the forms of all organized existences. In order, however, to accomplish this end, the following will first have to happen, namely, an abandonment of all hitherto accepted hypotheses of inheritance, a new conception of the nature of life, new views of the nature of the process of natural selection, and, above all, the abandonment of all such conceptions as gemmules, biophores, pangenes, plastidules, plassomes, etc., and the admission that the phenomena of life are ultimately physical in their nature and are to be treated in detail as physical problems.

It is now my firm conviction, also, that experimental investigation in embryology will make no solid progress until the foregoing prepossessions are abandoned, or until the mischievous influence of such speculations and their kindred, as those regarding a "germ-plasm," etc., have been entirely eradicated from the minds of the present generation. In conclusion, let me remark that five sciences are indissolubly connected together in the study of the fundamental problems of life; these are: physics, chemistry, physiology, morphology, and psychology. When each of these sciences shall have been given its due weight and place in the conduct of the study of life-forms, we shall begin to know what the latter really mean, but not until then.

FIFTH LECTURE.

ON THE NATURE OF CELL-ORGANIZATION.[1]

S. WATASÉ.

I.

If the true nature of a higher organism cannot be understood without considering the structure and the function of its component organs, it is equally certain that the nature of an individual cell cannot be made intelligible without a comprehensive study of the different organs of which it is composed.

At the present time, when morphologists are explaining the origin and development of the different structures in an organism in terms of cell-growth and of cell-metamorphosis, and when physiologists are referring the activities of the whole organism back to the functions of its component cells, it is natural that considerable attention should now be directed toward the solution of more elementary problems concerning the nature of the cell-organism. The cell-theory, while it explains the structure and functions of a tissue on a cellular basis, leaves the real nature of the cell itself unexplained.

The vital properties of a nucleated cell manifest themselves in various ways, but they may be broadly classified under two categories, *viz.*, (1), those tending to the preservation of the individual cell, and (2), those tending to the maintenance of the species. Under the first are included the general phenomena of cell-metabolism and different forms of irritability, and under the second those of cell-division and cell-fusion.

Diverse as are these special cell-phenomena which tend directly or indirectly to the preservation of the cell, it must

[1] Lecture given before the Biological Club of the University of Chicago, February 7, 1893, and afterward written out in the present form.

be admitted that their real significance can only be understood when each is taken in connection with the others, the *tout ensemble* of which form the life of the nucleated cell. If any explanation of special phenomena be attempted, it should be in the light of what we know of the whole.

We do not gain much, however, by so saying, as long as this knowledge of the whole is merely a name for the sum total of incoherent observations on diverse cell-phenomena. Beneath and beyond all vital manifestations of a cell, there must exist a primary physiological condition, on which the details of secondary cell-phenomena depend. Just what constitutes the primary physiological phenomenon of the cell, is the point that I wish to discuss in the present paper, believing, as I do, that such a problem as that of cell-organization, can be approached from the functional side with better effect than has thus far been attained from the side of pure morphology.

Before we proceed further, it may not be out of place here to introduce a general schematic description of a nucleated cell.[1]

Excluding the centrosome and chromatophore for the present, an animal cell may be described as composed of two sharply distinct organs: the cell-body (*cytosome*), and the nucleus (*caryosome*).

The nucleus, in its "resting stage," has a definite membrane around it, called the nuclear membrane or *caryotheca*. The cell-body consists of a net-work of *cytoplasm*. This net-work contains within its own substance small bodies of varying sizes, which are known as the *microsomes* or, more strictly, the *cytomicrosomes*. Surrounding the cytosome, there is a membrane known as the cell-membrane or *cytotheca*. It may exist as a thickened border of the cytosome or as a distinct membrane separated from the cytosome.

[1] The descriptive cytological terms adopted here have merely an anatomical significance, and do not refer to the chemical or functional properties of the structure. Several terms recently used by Hæckel (*Anthropogenie*, 4th edition, Leipzig, 1891), have therefore been found most convenient. Only the more salient features of the cell will be emphasized in this place, that being sufficient for our present purpose.

The meshes of the cytoplasm are filled with a fluid substance, commonly called the cytoplasmic fluid, or to use Hæckel's term, the *cytolymph*.

The cytoplasm is, however, the only living portion of the cell-body, and hence properly belongs to the category of *protoplasm* in the more strict technical sense of the term. The cytolymph is the inert, passive, non-living portion of the cell-body. Besides the cytolymph, there usually exist a number of non-living bodies in the cell-body, as yolk-granules, oil drops, débris of food, zymogen granules, etc., according to the nature of different cells. These non-living substances altogether belong to the group known as *metaplasm* or *paraplasm*, in contradistinction to the substance which is the real living element of the cell — the *protoplasm*.

The contents of the nucleus (*caryosome*) may be arranged also into two similar groups of living and non-living elements. The *chromosome* is distinctly protoplasmic in character, and so is the fine net-work of the "achromatic" thread-like substance which is often found traversing the nuclear cavity. In several cases, if not in all, these filaments are the actual continuation of the cytoplasmic net-work existing around the nuclear membrane.

The fluid substance which bathes these semi-solid living constituents of the nucleus is known as nuclear fluid or *caryolymph*. In the caryolymph there exists a body known as the *nucleolus*. In certain cases, the filaments of the chromosome have been found passing through the substance of the nucleolus or directly ending in it. Sometimes only one nucleolus exists in each nucleus, while in other cases over one hundred nucleoli may be found in one nucleus. The number of nucleoli is quite variable in different cells, but fairly constant in a given species of cell. The nucleolus is not a permanent body in the nucleus. It may exist at one stage of the cell, and may disappear at the next. The micro-chemical reaction of the nucleolus is entirely different from that of the chromosome. It appears probable that three or more different bodies are included under the same name of nucleolus. Indeed, one sees no reason why the inside of the nuclear membrane may not be used as a deposi-

tory for some solid products of cell-metabolism, under certain circumstances, just as the spaces in the cell-body are used for such a purpose. And thus *some* of the bodies included under the generic name of *nucleolus*, may belong to the group of metaplasm. It is, however, difficult to pass any definite opinion on the nature of the nucleolus at the present stage of our knowledge on the subject.

To recapitulate briefly, then, the chromosome and cytoplasm are the two *active*, living constituents of the cell. The rest of the bulk of a cell consists of non-living substances, which have yet to be converted into vital elements, or are the products of metabolism which have now lost the distinctive characteristic of a living substance.

The behavior of the cytoplasmic thread or network suggests that it is formed of a group of small, living particles, each with the power to assimilate, to grow and multiply by division. The chromosome, in the same way, is itself a colony of minute organisms of another kind, each endowed with similar attributes of vitality. The media, in which they live, — the cytolymph and caryolymph — are the media in which they breathe, from which they derive their nourishment, or within which they deposit the products of their metabolism. The reason why the cell as a whole assimilates, grows and divides, is ultimately due to the fact that the minute particles which compose the cytoplasm and chromosome are endowed with these functions.

Keeping in mind then such a simple nucleated cell like an amœba or an animal ovum, as a type, let us ask ourselves the following questions: What is the relation of nucleus[1] to cytoplasm, and of cytoplasm to nucleus in a cell? What is the significance of this duplex morphological organization? Through what process may such an organization as the nucleated cell be considered to have come into existence?

The biogenetic law as applied to the study of metazoan organisms, has been an important instrument of research in the field of comparative anatomy and embryology, but it is hard to

[1] In the following, the word "nucleus" is used, unless otherwise stated, synonymous with its essential constituent, the "chromosome."

use it as a working theory in the explanation of cellular phenomena such as I have already indicated. For it is possible that the law followed by the *cell-aggregate* in the course of its development, may not have been followed in the formation of the *individual cell*.

It is perfectly conceivable that the process by which the cell was first formed may have been due to causes of special character, while the conversion of the cell thus formed, into an organism of higher complexity, may have been due to causes entirely different from those that operated in the production of the nucleated cell.

There is no necessary reason to conclude, as has been done by several naturalists, that because the complex organism develops by a process of differentiation of the homogeneous germ, the duplex structure of the cellular units which compose the organism, must have also had a parallel course of development from its antecedent germ.

It is this point that I wish to discuss more in detail in the following.

II.

The parts of a living organism commonly termed its *organs* may be studied from three points of view : —

1. How far are these parts adapted, by their form and structure, to perform their physiological work? This mode of studying the organs belongs to physiology.

2. Where and how do they arise in a given organism? This field of study is a branch of morphology.

3. If the morphological study of the organ be extended through organisms of different grades of complexity, it may enable us to infer the probable steps through which the given organ may have passed in the course of its phylogenetic history.

When these three modes of study as pursued by naturalists at the present day, are applied to the study of organs of an individual cell, it resolves itself into three problems : —

1. How far are nucleus and cytoplasm adapted by their form and structure, to perform their physiological work in a given cell?

2. In what manner do they originate in a given cell? This ultimately resolves itself into the problem of cell-division (*Cytomery*) on the one hand and cell-fusion on the other.

3. What are the probable steps in the ancestral history, by which these structures came into existence? This belongs to the broad question of *Cytogeny* as understood in its phylogenetic sense.

In following out these questions more in detail, it is important to bear in mind at the outset, some vital distinctions involved in the use of the term *organ*, whether we understand it in a purely *physiological*, or in a *morphological* sense. From the purely physiological standpoint, any structure or member which, by its activity, contributes to the general welfare of the whole organism is an organ, whether that structure may have originated primarily in the organism, or may have been derived secondarily from an external source. Thus, the chromatophores[1] in the leaves of a plant are the organs of assimilation in that plant; the "gonidia" in the thallus of a lichen are the organs of a lichen, in a physiological sense as the heart or lung is the organ in the body of a higher organism.

But from the morphological side of the case it is different. According to the morphological view, *every differentiated organism, every organism composed of organs, can only have originated from a homogeneous stage by the differentiation of its parts.* To state this in another way, "however complicated one of the higher animals and plants may be, it begins its separate existence under the form of a nucleated cell. This, by division, becomes converted into an aggregate of nucleated cells: the parts of this aggregate, following different laws of growth and multiplication, give rise to the rudiments of the organs; and the parts of these rudiments again take those modes of growth and multiplication and metamorphosis which are needful to convert the rudiment into the perfect structure."[2]

[1] The term *chromatophore* (Schmitz) is here used in a broad sense, including leucoplasts and the various coloring substances in the flower and the fruit, as well as the chrophyll granules in the leaves of a green plant. The term is synonymous with Arthur Meyer's *trophoplast* and Schimper's *plastid*.

[2] T. H. Huxley: Article *Biology*, Encyclopædia Britannica, 9th edition, Vol. III, p. 682, 1878.

Now, this morphological criterion of an organ, which necessarily relates to the history and mode of its origin *within the organism, by the differentiation of its parts*, does not apply to the chromatophore in a green plant, nor to the "gonidia" in a lichen thallus, although there can be no doubt whatever that these structures serve as organs in the physiological sense, in the respective organisms. The chromatophores are not the products of differentiation of an homogeneous germ of the plant. They can only originate by the division of pre-existing chromatophores, if we follow such botanists as Schmitz, Schimper and Meyer.[1] The colorless protoplasm of the plant and the chromatophores are the coexistent but independent structures, with no genetic connection between them.

In the organization of a lichen, the case is clearer and more to the point of our inquiry. As is well known, the "gonidia" and their supporting meshwork are derived from two different groups of plants, *viz.*, *Algæ* and *Fungi*, respectively, although their physiological adaptation to each other is so perfect, so much so, in fact, that in several lichens the hyphæ of the fungus cannot live when separated from the algal portion, the "gonidia." Here, again, it is needless to say that these two organs are not the products of differentiation from some homogeneous *anlage* as different organs are in one of the complex animals.[2] On the other hand, the "gonidia" of one individual lichen are genetically related to the "gonidia" of another, and not at all to the hyphæ of the thallus. In a similar way the hyphæ of one individual lichen are genetically related to the hyphæ of another lichen of the same species, and not to

[1] Schmitz: *Die Chromatophoren der Algen*, Bonn, 1882. Schimper: *Ueber die Entwickelung der Chlorophyllkörner und Farbkörper*, Bot. Zeit., 1883, 41. Jahrg. Meyer: *Ueber Krystalloide der Trophoplasten und über die Chromoplasten der Angiospermen*, Bot. Zeit., 1883, 41. Jahrg.

[2] Before the discovery of the true nature of lichens, it was thought that both "gonidia" and supporting fungal hyphæ were the products of development of a single germinating spore. "Gonidia," which are the symbiotic algal cells, were supposed to be, as the term indicates, asexual organs of reproduction produced from the hyphæ and capable of development into a new and perfect lichen-thallus. The view that hyphæ might also be produced from the "gonidia" was often expressed. De Bary, *Historical Notice of the Lichens.* Comp. Morphology and Biology of Fungi, etc., p. 416.

the "gonidia," with which they live in close symbiotic relations.[1]

Thus the morphological and physiological consideration of an organ, such as we have just given, leads us to conclude that when we find two structures in an individual organism most intimately associated in their physiological relationship, it does not necessarily follow that they are organs in the morphological sense also. The history of the lichen clearly shows that *an independent organism, composed of organs, can be created by the union of two dissimilar organisms* by the establishment of an intimate physiological relationship between them. In fact, a certain number of species of lichens have been actually produced by bringing fungi and algæ together in a synthetic way.[2] The fungus and alga, by the interchange of their metabolic products, supply the nutritive wants of each, and thus produce the autonomous whole which can exist in places where neither the alga nor the fungus would be able to exist separately.

In dealing with the structures in an organism commonly called *organs*, I repeat, we must clearly bear in mind whether the structure in question is an organ in the physiological and morphological senses, or whether it is an organ simply in the physiological sense. If the structure is an organ in the morphological sense, the study of the development of the whole organism will show that it is a part of the products of differentiation of some preëxisting germ from which the entire organism was derived; if it be an organ in a purely physiological sense alone, there will be no genetic connection between the different structures, although each is indispensable to the existence of the other. In short, *the perfection to which the physiological adaptation of different organs is carried out in a given organism, is, in itself, no proof that they were derived by the differentiation of some common germ*; but, on the contrary, *two dissimilar organisms may, by mutual adaptation,*

[1] In using the term "symbiotic," to express the relation between the alga and fungus in the organism of a lichen, I simply follow such botanists as De Bary (*Die Erscheinung der Symbiose*, Strassburg, 1879, p. 15, et seq.), Frank (*Symbiose*, Lehrbuch der Botanik, Bd. I, 1892, Leipzig), and others.

[2] See, for example, the recent work by Bonnier, *Recherches sur la synthèse des lichens*. Ann. des sc. nat., 7me série, IX, Botanique, 1889.

give rise to a third organism in which each of them serves as an organ to the whole.

It is needless to say that almost all structures in higher organisms[1] are derived by the differentiation of some pre-existing germ, but at the same time it is important to bear in mind that this is not always the case, particularly in the lower forms. It is this consideration which interests us particularly, as it may be the key to the interpretation of the organization represented by the single nucleated cell.

III.

The permanent organs of the cell are, following the recent exposition of Strasburger,[2] considered to be (1) Cytoplasm, (2) Nucleus, (3) Centrosome, (4) Chromatophore.

The last named structure occurs normally in the cells of green plants, and sometimes in those of animals.[3] Following

[1] If Rückert's observation (*Über physiologische Polyspermie bei meroblastischen Wirbeltiereiern*, Anat. Anzeiger, Bd. VII, 1892) that in a certain vertebrate the merocyte-nuclei come from the nuclei of the supernumerary sperm-cells which enter the ovum, be confirmed, it would seem that a part of one important embryonic organ, in an organism like *Torpedo*, is bodily derived from the outside source.

[2] E. Strasburger: *Das Protoplasma und die Reizbarkeit*, 1891, Jena.

[3] See in this connection, Lankester's article *Animal Chlorophyll* (Nature, vol. 44, 1891) which is the review of G. Haberlandt's paper *Ueber den Bau und die Bedeutung der Chlorophyllzellen von Convoluta Roscoffensis*, in von Graff's *Die Organisation der Turbellaria acœla*, 1891, Leipzig. I have not been able to see Haberlandt's original paper. Haberlandt suggests, to quote Prof. Lankester, "that while phylogenetically they [chlorophyll-cells in *Convoluta*] must be regarded as Algæ (that is to say, have descended from Algæ) yet at the present time they have by profound adaptation to life in and with the *Convoluta*, altogether lost their character as algal organisms, and have become an integral histological element of the worm, and in fact constitute its assimilation tissue. . . . Haberlandt is inclined to place his theory as to the green cells of *Convoluta* alongside the suggestion of Schimper as to the origin of the chlorophyll corpuscles of higher plant — namely, that these are due to the union in the remote past of a green colored with a colorless organism."

Schimper has shown that the chromatophores (Schimper's plastids) are formed by the division of the preëxisting chromatophores, and not by the differentiation of the cell-protoplasm. Schimper's view referred to above on the origin of the chlorophyll bodies in the plant cells may be gathered from the following quotations: "Sollte es sich definitiv bestätigen," says Schimper, "dass die Plastiden in den Eizellen nicht neu gebildet werden, so würde ihre Beziehung zu dem sie enthaltenden Organismus einigermassen an eine Symbiose erinnern. Möglicher-

the views held by Schmitz, Schimper and others, we have already regarded this structure as an organ totally independent of the colorless protoplasm of the green plant. In regard to the centrosome, aside from its apparent function during the division of the nucleus, we know very little to justify our discussion in the present connection. Personally, I cannot agree with those who place the centrosome in the same category of the permanent cell-organs as the nucleus. On the other hand, I believe that the centrosome is a special form of the cytomicrosome, which exists almost in every part of the cell. For the reason of this homology of the centrosome, I may refer the reader to my former paper.[1]

weise verdanken die grünen Pflanzen wirklich einer Vereinigung eines farblosen Organismus mit einem von Chlorophyll gleichmässig tingirten ihren Ursprung." (Schimper: *Ueber die Entwickelung der Chlorophyllkörner und Farbkörper*, Bot. Zeit., 41. Jahrg., 1883, pp. 111–112.) Schimper supports this statement by quoting Reinke (Allg. Botanik, p. 62), who states that the chlorophyll bodies in the decomposing cells of a cucumber attacked by a certain fungus, still continued to grow and multiply.

In view of the fact that there exists a close analogy between the nucleus and the chromatophore (see Schmitz, *Die Chromatophoren der Algen*, Bonn, 1882, p. 167), the observations by Metschnikoff and Soudakewitch (*La phagocytose musculaire; contribution à l'étude de l'inflammation parenchymateuse.* Annales de l'Institut Pasteur, 6me Année, No. 1, Janvier, 1892, pp. 1–20, Pls. I–III), on the *repeated division of the muscle nuclei in the débris of degenerating muscle fibres which originally constituted a part of their cytoplasm, in the course of muscle degeneration*, may be interpreted in the same way as Schimper did of Reinke's observation on the behavior of the chlorophyll bodies in the decomposing vegetable tissue referred to above.

For the view that considers the animal chlorophylls as the veritable Algæ, see the well-known papers by Gesa-Entz and K. Brandt. Felix le Dantec, in his recent paper, *Recherches sur la symbiose des algues et des protozoaires* (Annales de l'Institut Pasteur, t. VI, No. 3, 1892), has brought further experimental evidence in support of the view that the chlorophyll corpuscles in an animal organism as *Paramœcium*, are the symbiotic Algæ. I mention these simply to call attention once more to the fact that certain parts of an organism, which were originally considered to be integral elements of the organism, derived by the differentiation of the germ, have been shown to be due, in reality, to a secondary association of two or more different organisms, originally separate and independent, and what we call *organs* from the physiological side, in such an organism, are in reality *organisms* by themselves.

[1] S. Watasé: *Homology of the Centrosome*, JOURNAL OF MORPHOLOGY, Vol. VIII, Pt. 2, 1893. A. Brauer (*Zur Kenntniss der Herkunft des Centrosomas*, Biol. Centralblatt, Bd. XIII, Nr. 9 u. 10, May, 1893), has recently come to the conclusion that in the spermatocyte of *Ascaris megalocephala*, variety *univalens*, the centrosome

If, therefore, I omit from consideration the chromatophore and the centrosome in the following, it is because the former has been fully treated by able botanists, and the latter is not sufficiently known, to my knowledge, to admit of useful discussion in connection with our subject. If, however, the centrosome be shown to be an organ of the cell, contrary to my former conclusion above alluded to, with a morphological significance comparable to that of the nucleus or the cytoplasm, the inference I advance in regard to the nature of the latter organs will apply equally well to the former.

Confining our remarks, then, to the nucleus and the cytoplasm, we may first ask whether the nucleus is to be regarded as an organ in a morphological sense, or only in a physiological one. Are nucleus and cytoplasm products of differentiation from some homogeneous *anlage* in the sense that all morphological organs are, or may not their constant occurrence in the cell rather be regarded as the result of a union formed in the remote past, between two organisms originally independent and dissimilar—a union of such a kind that ages of mutual adaptations have rendered their independent existence no longer possible? Is it not possible to regard the cell as a symbiotic community, in which the cytoplasm represents one group of

originates in the inside of the nuclear membrane. Brauer's view, however, does not militate against my statement that the centrosome is the cyto-microsome of a gigantic size, and that wherever cytoplasmic net-work exists there is a possibility of developing a microsome. When the centrosome originates inside of the nuclear membrane, it may be said, for a descriptive purpose, that it is derived from the nucleus; when it originates outside of the nuclear membrane, such a centrosome may be said to be cytoplasmic in its origin. Such a distinction is purely a nominal one, however, from my standpoint, and I believe the general statement that all centrosomes are cytoplasmic in their origin is fundamentally a correct one. Confusion only arises when we do not keep in mind the fact *that the cytoplasmic net-work, in the substance of which the microsome and centrosome arise, exists on both sides of the nuclear membrane, and the structure known as nucleus, contains a great deal of cytoplasmic substance in it.*

As is beautifully shown in Brauer's more recent paper (*Zur Kenntniss der Spermatogenese von Ascaris megalocephala*, Arch. f. mikr. Anat., Bd. 42, 1893, August, p. 185), the fact that in one variety of *Ascaris megalocephala*, namely, *univalens*, the centrosome lies inside, and in another variety, *bivalens*, outside of the nuclear membrane, is enough, to my mind, to show that it is the *substance* which gives rise to the centrosome, and not the *position* where the centrosome makes its first appearance, which we must consider in the determination of its homology.

extremely minute organisms, each with a power of growing, assimilating, and dividing; and the nucleus, or, more strictly, the chromosomes, a colony of still different forms, each with the same powers, — the whole making an organization comparable to that of the lichen, which is composed of two totally dissimilar organisms?

If this be so, then, there are two possible ways of explaining the nature of a nucleated cell; *viz.*, the *Theory of Differentiation*, such as was held by Hæckel, Auerbach, and several others, and more recently by Verworn [1] and Wiesner,[2] and the *Theory of Symbiosis*, as I have briefly suggested.

Let us examine the fundamental phenomena of the nucleated cell from the standpoint of the symbiotic theory, and, incidentally, point out the inadequacy of the differentiation hypothesis as an explanation of the cell phenomena in general.

IV.

Two important activities in the *developmental phases of protoplasmic life* [3] are cell-division, *e.g.*, caryokinesis, and cell-fusion, *e.g.*, fecundation. In both cases, the identity of the nucleus and of the cytoplasm is never once lost during the whole series of remarkable changes. There is a continuity of nuclear matter from one phase to another, just as there is a continuity of the cytoplasm through the successive periods in the history of the cell. In other words, the nucleus always originates from a preceding nucleus, and the cytoplasm from a preceding cytoplasm. There is no evidence proving that the nucleus is formed by the process of differentiation from the cytoplasm, nor that the cytoplasm is formed by the differen-

[1] Max Verworn: *Die physiologische Bedeutung des Zell-kerns*, Bonn, 1891, p. 115.

[2] J. Wiesner: *Die Elementarstructur und das Wachsthum der lebenden Substanz*, Wien, 1892, p. 266.

[3] Following the suggestion brought out by Professor Burdon Sanderson in his discourse on the elementary problems in physiology (*Nature*, Vol. XI, Sept. 26, 1889), it is convenient to divide the protoplasmic activities into two main groups — the *developmental* and the *non-developmental*. The former refers to those phenomena of the cell which are more especially connected with the development or unfolding of latent character of the germ, and the latter to such functions as respiration, secretion, excretion, etc.

tiation of nuclear substance. The developmental history of these two substances naturally leads us to regard them as independent structures, although each is necessary to the physiological existence of the other. They are not, therefore, morphological organs of the cell in the sense of the term as we have explained it. Furthermore, the phenomena of division in the nucleus and cytoplasm remind us forcibly of the mode of origin of the soredia in the lichen; nor is this remarkable, if a nucleated cell is, like the lichen, a symbiotic community of two dissimilar organisms. The single soredium is a miniature lichen, consisting of one or more algal cells with a weft of fungal tissue around them. The algal and fungal elements in a single soredium are derived from the corresponding elements in the mother lichen-thallus, just as the daughter cell derives its nucleus and cytoplasm from the corresponding elements in the mother cell.

The most convincing argument proving the symbiotic character of a lichen consists in the synthetic production of certain species of lichens, by bringing algal and fungal elements together. If, therefore, the morphological relationship between nucleus and cytoplasm in a cell is that of a symbiotic community, the fact analogous to the artificial synthesis of lichens must be found in the cell. I venture to suggest that such a synthesis of a living cell has been accomplished. I refer to Verworn's[1] experiment on the Radiolarian, *Thalassicolla*. Verworn took three vessels of equal size, and in the first he put a number of normal *Thalassicolla;* in the second, he put one which had its central capsule with its nucleus removed; in the third, he placed an individual whose central capsule had been removed and replaced by the transplantation of the central capsule of another individual of the same species. The Radiolarian in the third vessel was, therefore, a synthetic one, the extra capsular protoplasm lying outside of the central capsule, being derived from one individual, and the central capsule itself, with its nucleus, being derived from another. In the course of time, the *Thalassicolla* which had lost its nucleus by the removal of its central

[1] Verworn, *loc. cit.*, p. 42.

capsule died, while the other Radiolarian which had lost its nucleus and then regained it, by the acquisition of a central capsule of another individual, throve well, and could not be distinguished from the normal *Thalassicolla* in the first vessel, upon which no operation had been performed.

This is, in my judgment, a genuine case of synthesis of a living nucleated cell, by bringing, from two dissimilar sources, the cytoplasmic and nuclear elements together. If Stahl's and Bonnier's results, on the synthesis of lichens are the conclusive evidence of their symbiotic character, may not the Verworn's experiments on *Thalassicolla* be interpreted as equally conclusive evidence of the symbiotic origin of the nucleated cell? In fact, the process of fecundation is nothing more than a synthetic production of one nucleated cell from two nucleated cells that are derived from independent sources. And, further, there is good ground, as the phenomena of heredity show, for believing that each germ substance retains the individual qualities characteristic of its origin, throughout all stages of later development of the organism.

It is true that, in the case of a lichen, the algal and the fungal elements may exist independently, while in case of the nucleated cell, all investigation leads to the conclusion that when the nucleus is separated permanently from the cytoplasm or the cytoplasm separated from the nucleus, they invariably die, sooner or later.[1]

This is, however, no objection to the idea of the symbiotic origin of the nucleated cell, as a moment's reflection will show, for the more perfect the symbiotic adaptation of two organisms, the greater is their inability to live independently, until at last the symbiotic existence between the two organisms becomes imperative, if they are to continue to exist. Remove

[1] For recent contributions and a general summary of our knowledge on the relation between nucleus and cytoplasm, see O. Hertwig: *Die Zelle und die Gewebe*, Jena, 1893; E. G. Balbiani: *Nouvelles recherches expérimentales sur la mérotomie des infusoires ciliés.* Annales de micrographie, Nos. 8, 9 and 10, t. IV, 1892, Paris; Verworn: *Die physiologische Bedeutung des Zellkerns*, Bonn, 1891; Korschelt: *Beiträge zur Morphologie und Physiologie des Zellkernes*, Jena, 1889; Whitman: *The Seat of Formative and Regenerative Energy*, JOURNAL OF MORPHOLOGY Vol. II, 1888, Boston.

that opportunity, by one means or another, of their combining thus and death is the result, somewhat in the same way as in the case of an obligatory parasite, which though descended from a free-living ancestor, dies if deprived of its appropriate host.

The minute organisms which we assume to make up the cytoplasm on the one hand and nucleus on the other, probably once were free and lived independently, but if so, it is plain they have since lost their power by the acquisition of symbiotic habits. By adopting symbiotic habits, however, they acquired the ability to adapt themselves to surroundings so different from the normal habitat of each that neither symbiont alone could have lived in them, and thus possibilities each was incapable of accomplishing alone, are performed successfully by the composite organism.[1] We can reasonably suppose, therefore, that *those cell-forming organisms, which entered into the symbiotic relation in the past, with others, have survived in a modified form, in the body of the nucleated cell, while those organisms which did not, have perished owing to their inability to adapt themselves to the vicissitudes of circumstances.* The nucleated cell, then, is a colony of heterogeneous organisms, which maintains a complete autonomy and behaves as if it were an independent organic being, subject to the law of growth and development peculiar to itself.

It is perhaps needless to point out, after we have dwelt so much on the subject, that the fundamental assumption of our theory is that *at least some of the earliest living beings that ever existed were not in the form of a cell, but a great deal simpler, somewhat like those individual physiological units which constitute the cytoplasm and the nucleus of the cell.* The cell itself was formed later out of these still smaller organisms which already existed. It is not our purpose, in this place, to enter into an extensive examination of the different views that have been brought forward to explain what these cell-forming units are. All that is essential for our present purpose is the existence, as all writers unanimously agree, of such units in

[1] See important remarks on the result of commensalism between two organisms. Sachs: *Physiology of Plants*, pp. 391–394.

the cell, each with the power of assimilating, of growing and of multiplying by division.[1]

Perhaps the easiest way of arriving at the conception of the existence of such ultra-microscopic organisms in the cell is attained by separating the cytoplasm from the nucleus in a given cell, say an Amœba, and dividing each of them into smaller and smaller pieces, as far as our imagination can carry us. "Just as in division of the chemical mass we come to the chemical molecule, the further division of which changes the properties of the substance, so in the continual division of the Amœba we should come to a stage in which farther division interfered with the physiological action; we should come to a physiological unit, corresponding to, but greatly more complex than the chemical molecule."[2] Such a physiological unit, Foster suggests, might be called a *Somacule*. This physiological unit is what Weismann calls the "bearer of vitality," or *Biophor*, because it is the smallest unit which exhibits the primary vital forces., viz, *assimilation and metabolism*, *growth*, and *multiplication by fission*.[3] This unit is not the chemical molecule, hence it has all the essential characteristics of living organisms, such as assimilation and division, and mere molecules can neither assimilate nor multiply. It is, in fact, the organism itself, with all fundamental attributes of the higher organism; indeed, the reason why a higher organism exhibits these fundamental properties, is, as has already been mentioned, because its component units are endowed with such properties.

Although it is difficult to define the exact nature and morphological character of the physiological units individually, we can study them collectively in the phenomena of their *groupings*.

[1] The following are the examples of names recently proposed for the cell-forming units, by different writers: *Bioblasts* (Altmann, 1887); *Pangenes* (Hugo de Vries, 1889); *Somacules* (M. Foster, 1888); *Plasomes* (J. Wiesner, 1892); *Biophors* (Weismann, 1893); *Idioblasts* (O. Hertwig, 1893). Darwin's *Gemmules*, Nägeli's *Micellæ*, Spencer's *Physiological units*, Elsberg-Haeckel's *Plastidules*, Béchamp-Estor's *Microzymas* are already well known. It is important, however, to bear in mind that these names are not always synonymous.

[2] M. Foster: A Text-Book of Physiology, 5th edition, pp 5–6.

[3] A. Weismann: The Germ-plasma, pp. 39–40.

Take chromosomes of the nucleus, for example. In some cells the physiological units of the chromosome arrange themselves in a series of short rods — chromatomeres — at a certain period of their existence, while in others they arrange themselves in a series of elongated filaments at the corresponding period. What, therefore, we see in the grouping of micro-organisms such as bacteria, exactly applies to those units which form the chromosomes. As De Bary[1] says of bacteria, so we may say here, that it is precisely in the phenomena of grouping that specific peculiarities of conformation of such physiological units best display themselves, being collected together, as it were, in large quantity. These groupings are forms of vegetative development, *growth-forms* of the minute organism which constitutes the physiological unit of the chromosome.

The growth-forms of the physiological unit forming the cytoplasm of the cell are equally characteristic, as is plainly shown in the reticular, striated, or fibrillar arrangements of the cytoplasm in different kinds of cells.

V.

To re-state the problem, then, we may say that there exist two easily recognizable elements in every cell, *viz.*, (*a*) the nucleus, or more strictly the chromosome, and (*b*) the cytoplasm.

It has been conclusively settled by a number of investigators that the presence of both is essential for the continued manifestation of life activity in a given cell. A piece of cytoplasm or nucleus may continue to maintain a certain activity after it has been detached from the cell, but it perishes, sooner or later, if left alone by itself.

So much is certain, but how and why each is essential to the other are quite other questions, and have received different answers from different investigators. Some claim that the nucleus influences the cytoplasm by some dynamical action; others hold that some invisible living particles of the nuclear matter diffuse through the nuclear membrane, and become converted into the substance of the cytoplasm; while still

[1] De Bary: *Lectures on Bacteria*, Oxford, 1887.

others believe that the influence of the nucleus upon the cytoplasm is that of a fermentative action. To a fourth group of investigators, (*e.g.*, Verworn), again, the action of nucleus and of cytoplasm is a reciprocal one, the nucleus influencing the cytoplasm, and the cytoplasm influencing the nucleus in return, by the interchange of the metabolic products. This last view is adopted in the present paper. The results of operations performed on the nucleated cell seem best to support this view.

All of these explanations are finally reduceable to two fundamentally different views one may take in regard to the nature of chromosome and cytoplasm in each cell. Some hold (*a*) that the nucleus and cytoplasm are essentially one and the same substance, and that they only differ from each other, in so far as their stages of development are concerned. Hence the difference between the nucleus and the cytoplasm is merely that of degree. Others, on the other hand, maintain (*b*) that the difference between the nucleus and the cytoplasm is not that of a degree of development of one and the same substance, but of the kind of material of which each is composed.

So far as we can judge from the micro-chemical reactions of nucleus and cytoplasm, they must be regarded as belonging to two substances entirely different from each other. There is no evidence to show that one is actually produced from the other, but the cytoplasm always originates from the preceding cytoplasm, and the nucleus always from the preceding nucleus.

There are, then, four well ascertained facts, in regard to the nature of nucleus and cytoplasm ; *viz.*,

1. The two elements in each cell — the chromosome and the cytoplasm — have the capacity for assimilation, growth and multiplication by division.

2. Each is essential to the physiological existence of the other.

3. The chromosome always originates from the preceding chromosome, and the cytoplasm from the preceding cytoplasm.

4. Each has a definite micro-chemical reaction, different from the other, and is composed of different chemical substance.

Any theory that explains the nature of a nucleated cell must explain all of these, and hold them under one common point of view. *Such a theory must, in short, recognize not only the profound physiological interdependence between nucleus and cytoplasm, but it must also recognize their natural morphological independence.*

The doctrine of symbiosis, first propounded by De Bary,[1] just fulfils these requirements, inasmuch as it means now, in a more restricted sense, *the normal fellowship or the consortial union of two or more organisms of dissimilar origin, each of which acts as the physiological compliment to the other in the struggle for existence.*

Under the assumption of such a principle as that of mutualistic symbiosis, the fact of natural anatomical difference between the chromosome and the cytoplasm can only be harmonized with the fact of their complimentary physiological adaptation. Only on the assumption that the chromosome and cytoplasm had dissimilar *origin*, can we understand their constant difference in optical, microchemical and anatomical characters, through all phases of their activity.

To summarize for the sake of clearness, then, the general consequence of the symbiotic existence to its participants, we may say, that, (1) inasmuch as one organic being comes in connection with another in order to be nourished and nourish the other in return, they obtain a freedom in the choice of dwelling place, which is not enjoyed by them otherwise; (2) symbiosis of two dissimilar organisms induces certain modification in each symbiont, by the suppression of certain characters originally present in each, or by the acquisition of others, which were formerly absent; (3) when the adaptation of one symbiont to the other becomes perfect, the whole community behaves like a new organism, subject to new laws of growth and of development, and is no longer subjected to those relating to each symbiont separately, and thus, (4) a power of adaptation to the external world which each symbiont did not possess individually, in the struggle for existence, may be acquired indirectly, by the combined efforts of the two. (5)

[1] De Bary: *Die Erscheinung der Symbiose*, Strassburg, 1879.

In proportion as the symbiotic adaptation of two or more organisms becomes more and more perfect, each symbiont loses the power of living independently which it originally possessed.

The view that ascribes a symbiotic significance to the association of these two different kinds of cell-forming organisms in each cell, explains the following points, *viz.*, (I) the constant difference in anatomical, optical and micro-chemical characteristics between the chromosome and the cytoplasm; (II) the maintenance of their specific identity through all phases of *developmental* changes, as caryokinesis and fecundation; (III) the participation of both nucleus and cytoplasm in the manifestation of *non-developmental* phenomena of cell-life, such as secretion, excretion, etc.; (IV) the interchange of metabolic products between nucleus and cytoplasm as the necessary outcome of a symbiotic mode of existence; (V) the reason why the cytoplasm separated from the nucleus, or the nucleus isolated from the cytoplasm invariably perishes; and, therefore, (VI) why the nucleus and cytoplasm are the physiological organs of a cell, and yet they are not organs from a morphological or developmental standpoint.

The nuclear substance must not be considered, in any sense, as inactive, which becomes only active when it migrates into the cytoplasm as Hugo de Vries[1] maintains in his well-known work. The nuclear substance of a cell is just as much active as the cytoplasm, according to the present view, but in an entirely different manner, somewhat in the same way as the chlorophyll-bearing algal cells and the colorless fungal elements in a lichen are active at the same time, but each in its own way.

The vital properties of a cell do not reside in the nucleus alone, nor in the cytoplasm which surrounds it, but in the two together. The cell may be destitute of a cell-wall — *cytotheca* — or each may be enclosed within its own cell-membrane, or a cell may exist side by side without any visible boundary between them — the syncytium. The division of an organism into distinct cell-entities in a multicellular organism is a phenomenon widely distributed, it is true, but still of secondary

[1] Hugo de Vries: *Intracellulare Pangenesis*, Jena, 1889.

significance,[1] due to physiological causes, I believe, emanating from the fundamental difference existing between the chromosome and the cytoplasm, — the difference between the two being of such a character that makes their mutual association necessary for the existence of each. The chromosome cannot grow beyond a certain bulk, nor is the cytoplasm capable of unlimited growth, without each meeting with restraining influence from the other, if one may express it in a metaphorical way. The formation of a nucleated cell is, in other words, a secondary adaptation to keep the nuclear and cytoplasmic material within the reach of reciprocal physiological influence of each. The division of the cell, when such exists, is the result incidental to the increase in the number of two kinds of cell-forming organisms existing in each nucleated cell.

The sphere within which the symbiotic reciprocal influence of these two cell-forming organisms is felt, corresponds to what Sachs[2] calls the *energid*. The term *energid* as a substitute for the modern idea of the *nucleated cell*, aptly expresses one aspect of the cell-organism, namely, its physiological side. From the genetic standpoint, as given in the present paper, this single *energid* is already a complex of at least two kinds of organisms, different in their anatomical character, in their function, and in their origin.

Stated in this way, the view is not a new one, but agrees, in its broadest feature, with the idea of a cell expressed by Darwin in his theory of Pangenesis,[3] and in its special aspect which considers mutualistic symbiosis as the basis of cellular organization, may perhaps add a more concrete meaning to his well-known passage, without necessarily adopting his further inference from it, when he said that "an organic being is a microcosm — a little universe, formed of a host of self-propagating organisms inconceivably minute and numerous as the stars in the heavens."

[1] Sachs: *Lectures on the Physiology of Plants*, p. 73.

[2] Julius v. Sachs: *Physiologische Notizen II. Beiträge zur Zellentheorie.* Flora: Jahrg. 75, 1892. Reprinted in his *Gesammelte Abhandlungen, II.*, 1893. pp. 1150–1155.

[3] Darwin: *Provisional Hypothesis of Pangenesis* (*Animals and Plants under Domestication*, vol. ii.); *The Descent of Man*, Appleton, p. 228.

SIXTH LECTURE.

THE INADEQUACY OF THE CELL-THEORY OF DEVELOPMENT.[1]

C. O. WHITMAN.

THE doctrine of Schleiden and Schwann that in *cell-formation* lies the whole secret of organic development, has held the place of a central axiom in biological work and speculation for over half a century. All this time the cell has been, as it were, the alpha and omega of both morphological and physiological research. Regarded as a primary element of structure, it has come to signify in the organic world what the atom and molecule signify in the physical world.

The traditional *cell-standpoint* has been most exactly defined by Schleiden and Schwann. In his celebrated "Beiträge zur Phytogenesis" (Müller's Archiv, 1838), Schleiden sets forth the cell-doctrine, which he limited to plants, in the following words: "*Each cell leads a double life; an independent one, pertaining to its own development alone; and another incidental, in so far as it has become an integral part of a plant.*"

Schwann, in his classical Researches of 1839, extends the same view to the entire organic world.

"*Each cell,*" he affirms, "*is, within certain limits, an individual, an independent whole. The vital phenomena of one are repeated, entirely or in part, in all the rest. These individuals, however, are not ranged side by side as a mere aggregate, but so operate together, in a manner unknown to us, as to produce an harmonious whole.*" (Introduction, p. 2.)

"*The whole organism subsists only by means of the reciprocal action of the single elementary parts.*"

The method of reasoning is precisely the same as we have seen in some of the latest experimental studies on cleavage. Witness the following: "If we find that some of these

[1] Read Aug. 31, at the Zoölogical Congress of the World's Columbian Exposition.

elementary parts, not differing from the others, *are capable of separating themselves from the organism*, and pursuing an independent growth, we may thence conclude that each of the other elementary parts, each cell, is already possessed of power to take up fresh molecules and grow; and that, therefore, *every elementary part possesses a power of its own, an independent life, by means of which it would be enabled to develop independently*, IF THE RELATIONS WHICH IT BORE TO EXTERNAL PARTS WERE BUT SIMILAR TO THOSE IN WHICH IT STANDS IN THE ORGANISM. The ova of animals afford us examples of such independent cells, growing apart from the organism." (*l. c.* p. 192).

In these words of Schleiden and Schwann we see no vague anticipation, but a clear statement, of the cell-standpoint of to-day. The organism consists, morphologically, of cells, and subsists, physiologically, by means of the "reciprocal action" of the cells. *Organization* means cellular structure, and *ontogeny* means cell-formation. "*Der gleiche Elementarorganismus ist es, der Thiere und Pflanzen zusammensetzt.*" (Schwann.)

In this "double life," this "harmonious whole," this "reciprocal action" of "elementary organisms," this "operating together in an unknown manner," we see the "cell-state" theory, the "unknown principle of correlation," the "correlative differentiation," the "cellular interaction" of current literature.

Much as we have enlarged our knowledge of the cell, we are still looking at the problems of life from the point of view occupied by the founder of the cell-doctrine. The most notable advances in cytology have but tended to define and emphasize the cell-standpoint. The discovery that *all cells arise by division of prëxisting cells*, neatly embodied in Virchow's maxim, "*omnis cellula e cellula*"; the extension and verification of this maxim furnished by Gegenbaur in 1861, in demonstrating *the vertebrate egg to be a single cell*; and the proof obtained during the last twenty years that the *internal processes of cell-division are fundamentally the same in both plants and animals*, — all these capital steps forward have tended to magnify the importance of the cell as a universal unit of structure.

All higher organization is supposed to begin with cell-formation, and to reach its fullest expression in the mutuality of the constituent cells. Whether the cytoplasm be regarded as isotropic or as definitely organized, whether the hereditary substance be identified with the egg as a whole, or with the nuclear chromosomes alone, the cell-dogma is still supreme.

Our microscopes resolve the organism into cells, and ontogeny shows that the many cells arise from one cell; hence, the organism seems to be the product of cell-formation, and the cleavage of the germ seems to be a *building* process. The cell-theory points us to very definite units, as the elements of organization, and thus offers what has for a long time appeared to be a rational basis for the investigation of life-phenomena. All the search-lights of the biological sciences have been turned upon the cell; it has been hunted up and down through every grade of organization; it has been searched inside and out, experimented upon, and studied in its manifold relations as a unit of form and function. It has been taken as the key to ontogeny and phylogeny, and on it theories of heredity and variation have been built. For a long time it has been regarded as a decisive test of homology in germ-layers, tissues, and organs. Fundamental distinctions have been made between *intra*-cellular and *inter*-cellular organization, between unicellular and multicellular organisms and organs, between cellular and acellular growth and development, between the processes of fission and regeneration in the protozoan and the metazoan, between differentiation *within* the cell and *among* cells, between the formative forces which shape the infusorian and those which act in a many-celled organism.

An organism of many cells is supposed to differ from one of one cell, somewhat as a complex molecule differs from a simple one. The complex unit bears not only the structure of its individual parts, but also a totally new structure formed by the union of these parts. In like manner the organism is fancied to carry at least two distinct organizations, the organization of the separate cells and that of the cells united. The higher organization thus differs, *qualitatively*, from the lower, so that

we may have analogies, but no homology of organs between unicellular and multicellular organisms.

How sharply the line is drawn in this regard is shown in the scrupulous care with which authors avoid the suggestion of anything comparable to muscle or nerve in the infusorian. The Ehrenberg view of infusorian organization demanded altogether too much, and we have swung to the opposite extreme of thinking that the very idea of such comparison is forbidden by the cell-doctrine. Any suggestion of a possible community of origin between an organ — say the *mouth* — of such an animal and the corresponding structure of a cellular organism, would be quickly relegated to the *limbus fatuorum*. Who dares question the proposition that there *can* be no morphological identity between an organ formed without cells and one formed with cells? No matter how complete the physiological correspondence, the two things must be assumed to differ *toto coelo*, as measured by the cell-rule. That is the cell-standpoint.

While the cell-doctrine has been carried steadily forward, confidence in its all-sufficiency has been somewhat shaken from time to time, and a few cautious protests have been ventured against the complete ascendancy of the cell as a unit of organization. Botanists, among whom in this particular the name of Sachs stands foremost, have led the way to another standpoint, which, in contradistinction to the prevailing one, may be called the *organism-standpoint*. Among zoölogists, Rauber has most boldly and ably defended this point of view; and more recently Wilson has expressed similar views, but with reservations that still uphold the cell-standpoint. Driesch, too, obtains experimental proof that "the *mode* of cleavage is something unessential to the future animal," but still he feels compelled to explain the organism from the cell-standpoint, — that is, he supposes that the organism is determined by *correlative* differentiation of homodynamous ("omnipotent") cells or nuclei. The position is altogether similar to that of Oscar Hertwig and Wilson. Wilson, however, holds that the cleavage may secondarily acquire a "mosaic" significance, and herein makes a decided advance towards a pre-organization

theory. *A certain grade of organization as the result of heredity* rather than of cleavage is conceded for annelid development, and for all forms, in so far as future characters are foreshadowed in cleavage stages. This is a limited application of the view which I believe holds true of all eggs, *even before cleavage begins*. It will be easy to show that the very facts generally relied upon to disprove the existence of organization in the egg furnish very strong evidence in support of it.

The question as to the presence of organization is not settled by the *form* of cleavage. Eggs that admit of complete orientation at the first or second cleavage, or even before cleavage begins, are commonly supposed to reflect *precociously* the later organization, while eggs, in which such early orientation is impossible, are supposed to be more or less completely isotropic and destitute of organization. When the region of apical growth is represented by conspicuous teloblasts, the fate of which is seen to be definitely fixed from the moment of their appearance, we find it impossible to doubt the evidence of organization, or "precocious differentiation," as it is conventionally called. When the same region is composed of more numerous cells, among which we are unable to distinguish special proliferating cells, we lapse into the irrational conviction that the absence of definitely orientable cells means just so much less organization.

Cell-orientation may enable us to infer organization, but to regard it as a measure of organization is a serious error. The organization of a vertebrate embryo cannot be said to be less advanced than that of an annelid embryo, because it lacks the unicellular teloblasts which the latter may possess. The regular holoblastic cleavage of the mammalian egg is evidently no index to its grade of organization. The more carefully we compare the cleavage in different eggs, the more clear it becomes that the test of organization in the egg does not lie in its mode of cleavage, but in subtile formative processes. We find the most unlike forms of cleavage issuing in the same remarkable form-phases ; for example, the primitive streak of mammalian and avian eggs ; and conversely, we find identical forms of cleavage leading to fundamentally different

results ; for example, in the egg of the polyclad as compared with that of the mollusc or the annelid, where "*cells having precisely the same origin in the cleavage, occupying the same position in the embryo, and placed under the same mechanical conditions, may nevertheless differ fundamentally in morphological significance.*" (Wilson.)

The most remarkable feature of avian development is the primitive streak. The presence of this feature in typical form, in such an egg as that of the mammal, is certainly one of the most significant facts in embryology. The conclusion is here forced upon us — and I see no escape from it — that the formation of the embryo is not controlled by the form of cleavage. The plastic forces heed no cell-boundaries, but mould the germ-mass regardless of the way it is cut up into cells. That the forms assumed by the embryo in successive stages are not dependent on cell-division, may be demonstrated in almost any egg. Watch the expansion of the blastoderm in the pelagic teleost egg, the formation of the germ-ring, and especially the *axial concentration of material,* which is so beautifully illustrated in these eggs. Such developmental processes are, if I mistake not, clearly indicative of some sort of organization.

The formation of the whole from a part, regarded by some as conclusive evidence of isotropy and correlative *cell*-differentiation, no more disproves the existence of definite organization in the case of the egg than in the case of hydra. A fragment of a hydra may reproduce the whole organism ; and in so doing act as a unit, not as a fraction of a unit. In the same way, one of the first two or four blastomeres, when severed from vital connection with its fellow or fellows, may develop *as a unit, not as a half-unit,* precisely as Wilson insists is the case in Amphioxus.

If the isolated blastomere continues for a while to form cells as if it were a half-unit or a quarter-unit, and only later manifests the whole unit-power of the organism, I see no reason to conclude that the case is fundamentally different. In either case the part has the power of reorganizing itself into the whole, and it makes no essential difference whether the reor-

ganization be accomplished at once, before cells are formed, or gradually, while cell-formation is going on.

If we no longer hesitate to accept Brücke's view that the functions of the cell are proof of organization, although our best microscopes fail to give us any idea of what it consists in, it certainly ought not to be difficult to regard the egg as a young organism, and the developmental phenomena as proof of organization. Such organization is, in fact, conceded when we speak of the egg as the rudiment of an organism ("Anlage eines Organismus," O. Hertwig), but, nevertheless, we go on insisting that cellular structure is the essence of a higher organization.

We are so captured with the personality of the cell that we habitually draw a boundary-line around it, and question the testimony of our microscopes when we fail to find such an indication of isolation. We have so long insisted on these boundary-lines as limiting homologies that we find it extremely difficult to ignore them. How difficult it is, for example to regard a multicellular nephridial funnel as the exact homologue of the unicellular funnel. If the organ consist of one cell, the tube is *intra*-cellular; if of many cells, then it is *inter*-cellular. But we have the "tube" and the "flame" just as perfect with one cell as with many, as Vejdovsky's studies make very certain. How idle, then, to deny homology between two such organs merely because one is *intra*- and the other *inter*-cellular. And yet that is precisely what we have been accustomed to do.

Now this one case illustrates, as I believe, a general truth of no little importance. *The nephrostome is a nephrostome all the same whether it consist of one cell, two cells, or many cells. Its form and function are both independent of the number of component cells. Cells multiply, but the organ remains the same throughout. So far as homology is concerned, the existence of cells may be ignored.*

May we not go further, and say that an organism is an organism from the egg onward, quite independently of the number of cells present? In that case *continuity of organization* would be the essential thing, while division into cell-territories might

be a matter of quite secondary importance. As the nephrostome is not the result of cell-formation, but exists as such before division into cells, so the organism exists before cleavage sets in, and persists throughout every stage of cell-multiplication. Continuity of organization does not of course mean preformed organs, it means only that a definite *structural* foundation must be taken as the starting-point of each organism, and that the organism is not multiplied by cell-division, but rather continued as an individuality through all stages of transformation and sub-division into cells.

We have long been aware that the cell could not be taken as the ultimate unit of life, and every notable effort to account for heredity has led to the postulation of primary elements in comparison with which the cells appear as complex organisms. Since Ernst Brücke first contended for the organization of the cell in 1861, and the existence of "smallest parts" as the basis of this organization, we have seen similar ideas reappear in the "physiological units" of Herbert Spencer, the "gemmules" of Darwin, the "micellae" of Nägeli, the "plastidules" of Elsberg and Haeckel, the "inotagmata" of Th. Engelmann, the "pangens" of de Vries, the "plasomes" of Wiesner, the "idioblasts" of Oscar Hertwig, and the "biophores" of Weismann.

After the discovery of cell-division as the law of cell-formation, and after the scheme of the cell set up by Schleiden and Schwann had been revised and reduced to essentialities by Leydig, Max Schultze, and others, the next great step forward in the cell-doctrine must be credited to Brücke, who, seeing that the phenomena of life could not be referred to a *structureless* substance, declared for the *organization* of the cell in words that were scarcely less than revolutionary.

"We must therefore," says Brücke, ascribe to living cells, in addition to the molecular structure of the organic compounds which they contain, still another, and otherwise complicated, structure; and this it is that we designate by the name organization."

Further, in his own words: "*Wir müssen in der Zelle immer einen kleinen Thierleib sehen, und dürfen die Analogien, welche zwischen ihr und den kleinsten Thierformen existiren, niemals aus den Augen lassen.*" (Elementarorganismen, p. 387.)

On the botanical side, Sachs has maintained since 1865 ("Experimental-Physiologie") that protoplasm is an "*organized body*" (*cf.* Lectures on Physiology, 1887, p. 206–7). While Brücke contended for organization *within* the cell, and remained true to the cell-theory of all higher organization, Sachs, Goebel and some other botanists early challenged the doctrine of cell-hegemony. Sachs briefly indicates his standpoint in the following words :

"To many, the cell is always an independent living being, which sometimes exists for itself alone, and sometimes 'becomes joined with' others — millions of its like, in order to form a cell-colony, or, as Haeckel has named it for the plant particularly, a cell-republic. To others again, to whom the author of this book also belongs, cell-formation is a phenomenon very general, it is true, in organic life, but still only of *secondary significance;* at all events, it is merely one of the numerous expressions of the formative forces which reside in all matter, in the highest degree, however, in organic substance." (Lectures, etc., p. 73.)

Brücke's great merit consists in this, that he taught us the necessity of assuming *structure* as the basis of vital phenomena, in spite of the negative testimony of our imperfect microscopes. That function presupposes structure is now an accepted axiom, and we need only extend Brücke's method of reasoning, from the tissue-cell to the egg-cell, in order to see that there is no escape from the conclusion that the whole course of developmental phenomena must be referred to organization of some sort. *Development, no less than other vital phenomena, is a function of organization.*

Nägeli followed the same method of reasoning when he concluded that the organism was, in a certain sense, "vorgebildet" in the germ-cell (Beiträge zur wiss. Botanik, Heft II. 1860). This point of view is well expressed in his classical work, the "Theorie der Abstammungslehre," where he says: "Organisms differ from one another as egg-cells no less than in the adult state. The species is contained in the egg of the hen as completely as in the hen, and the hen's egg differs from the frog's egg just as widely as the hen from the frog."

While all will admit that the organization of the egg is such as to predetermine the organism, few will be prepared to admit that *the adult organization is identical in its* INDIVIDUALITY

with that of the egg. The organism is regarded rather as a community of such individualities, bound together by interaction and mutual dependence. According to this view, development does not consist in carrying forward continuous changes in the same individual organization, but in multiplying individualities, the complex of which represents, at every stage, not *the* organism, but one of an ascending series of organisms, which is to terminate in the adult form.

In the egg-cell we are supposed to have an *elementary* organism ; in the two-cell stage, two elementary organisms, forming together an organism of a totally different order, based on a new scheme of organization. In the four-cell stage we have another organism, in the eight-cell stage another, and so on.

"Physiological division of labor," as Milne-Edwards first phrased it, is unquestionably a principle of wide application. Given the cells as morphological units and this physiological principle, the evolution of a cellular organism, may be conceived of as a most simple affair. From a simple colony of like cells, we pass to a commonwealth of differentiated and mutually dependent cells. A multitude of independent cell-organisms, adopting mutual service as the best economy, find themselves in the end incapable of independent life, and so firmly bound together in interdependence, that they constitute a complex individual. The usual conception of this division of labor is, as Herbert Spencer[1] has recently stated it, "an *exchange of services*, — an arrangement under which, while one part devotes itself to one kind of action, and yields benefits to all the rest, all the rest, jointly and severally performing their special actions, yield benefits to it in exchange. Otherwise described, it is a system of *mutual* dependence."

We habitually apply this anthropomorphic conception to every grade of organization. The higher organism is regarded as a colony of cells ; the cell as a colony of simpler units, nucleus, centrosome, and so on ; the nucleus as a colony of chromosomes ; the chromosome, according to Weismann's terminology, as a colony of "ids"; the "id" as a colony of

[1] The Contemporary Review, February, March, and May, 1893.

"determinants"; the "determinant" as a colony of "biophores," and the "biophore" as a colony of molecules.

In proportion as division of labor is carried out, *interdependence* is increased, and the units become more and more intimately associated. The struggle for existence is supposed to extend to the cells, and even to the biophores. Symbiotic relations are fought out, refined, and confirmed by natural selection, and eventually reduced to a system of mutual adaptations which are fancied to be the basis of organic unity.

Whether organization is wholly a matter of acquisition, and whether it became possible only as a result of symbiotic advantages accidentally discovered in the struggle for existence, need not here be discussed. It is enough for present purposes to know that organization exists, and that *organic* unity depends on *intrinsic properties* no less than does *molecular* unity.

It is not division of labor and mutual dependence that control the union of the blastomeres. It is neither functional *economy* nor social instinct that binds the two halves of an egg together, but the constitutional bond of *individual organization*. It is not simple adhesion of independent cells, but integral structural cohesion.

That organization precedes cell-formation and regulates it, rather than the reverse, is a conclusion that forces itself upon us from many sides. In the infusoria we see most complex organizations worked out within the limits of a single cell. We often see the formative forces at work and structural features established before fission is accomplished. Cell-division is here plainly the result, not the cause, of structural duplication. The multicellular Microstoma behaves essentially in the same way as the unicellular Stentor, or the multinucleate Opalinopsis of Sepia. The Microstoma organization duplicates itself, and fission follows. The chain of buds thus formed bears a most striking resemblance to that of Opalinopsis, and the resemblance must lie deeper in the organization than cell-boundaries.

Compare the results obtained by artificial division in two such forms as Stentor and Hydra. The two courses of regen-

eration are so exactly parallel that one cannot fail to see at once that the formative forces operate in essentially the same manner with the one-celled as with the many-celled organism. Gruber's experiment, as described in his recent article, "*Microscopic Vivisection*" (Berichte der Naturforschenden Gesellschaft zu Freiburg, Vol. VII, Part 1, 1893), illustrates well this point.

A Stentor was cut into three pieces, *A*, *B*, *C*, each of which regenerated the missing parts within 24 hours. The anterior

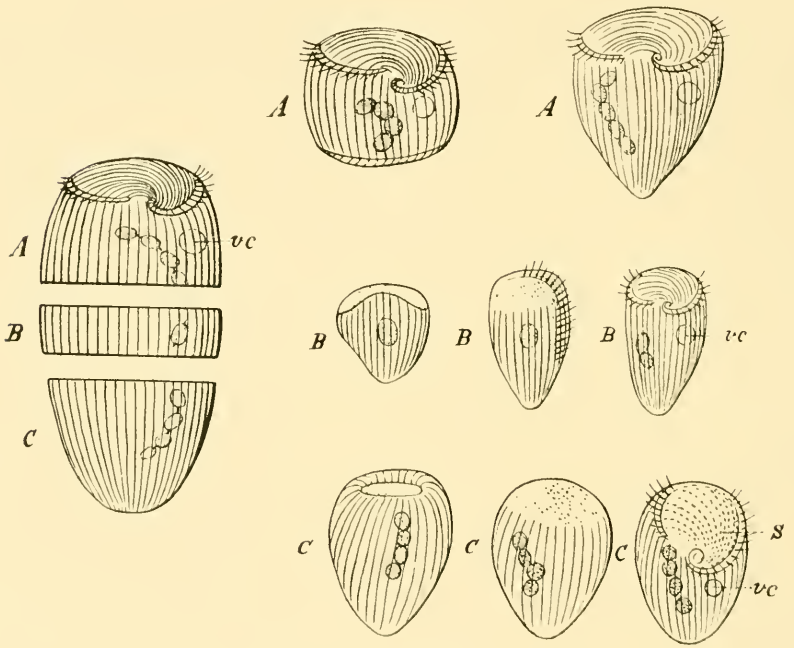


FIG. 1. — Regeneration of a Stentor cut into three parts, *A*, *B*, *C*. *vc* = pulsating vacuole. *S* = regenerating frontal field.

end regenerated posterior end, and *vice versa*. The middle piece regenerated both ends — the complicated frontal field with its mouth, pharynx, long cilia, pulsating vesicle, *etc.*, as well as the simpler posterior region.

Treat a Hydra in the same way and similar results will follow. In both cases the orientation of the parts will remain the same as that of the whole. Gruber repeated the division of Stentor four times in succession, getting perfect regeneration each

time, but *smaller* individuals, as no growth was possible. The experiment reminds one of the half- or quarter-sized embryos obtained by separating the first two or four blastomeres.

Gruber's highly interesting paper calls attention to the identity in form and structural detail of the "membranellae" of Stentor with the so-called "corner-cells" (Eckzellen) of molluscs (*Cyclas cornea*). The comparison is a most instructive one, illustrating in the most conclusive manner that differentiation of the parts of the *soma* depends, not on the interaction of cells, but upon the elementary structure of the protoplasm.

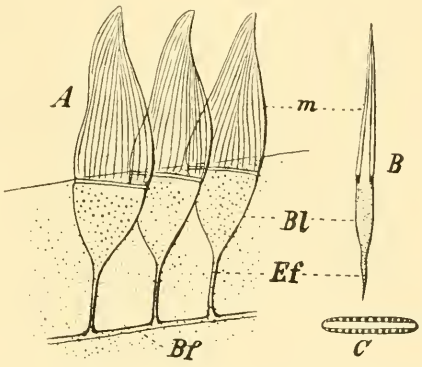


FIG. 2. — *A*, three membranellae of Stentor. *B*, membranella in section. *C*, Section at the base of the two plates. *Bl*, Basal lamella. *Ef*, Terminal fibre. *Bf*, Basal fibril. *K*, Nucleus.

The membranellae of the frontal field of Stentor consist of two thin, adherent plates, each of which represents a number of coalesced cilia. The structure has a basal seam or ridge[1] (Leiste), and a basal lamella which is continued into a terminal fibre. All these fibres are connected by the basal fibril, through which the movements of the membranellae are evidently regulated.

Now this highly differentiated organ, the membranella, is reproduced with most remarkable exactness in the "corner-cell" of Cyclas. But here the organ represents an individual

[1] This seam consists of a series of microsomes, as Dr. Watase has discovered.

cell, while in Stentor a whole crown of such organs is formed without any division into cells. Could one ask for a clearer demonstration? Are we not forced to conclude with Gruber that "*however great the difference between an infusorium and a highly organized animal, it cannot be a qualitative one. We can assume that the same vital elements serve in both as the foundation, only in ever new combinations. This kinship declares itself very clearly in the correspondence of many organs of the infusoria with those of the higher organisms*" (*l. c.* p. 16).

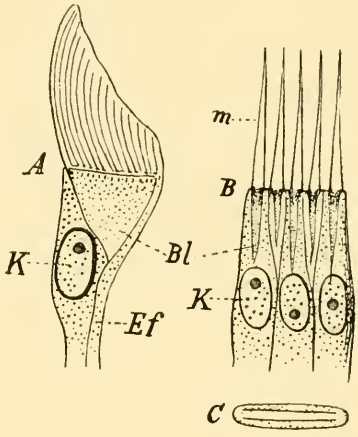


FIG. 3. — *A*, "Corner cell" of *Cyclas cornea*. *B*, Section of three cells. Other letters as in Fig. 2.

"So finden wir," says Gruber, "in einem Thiere, das schon hoch auf der Stufenleiter der vielzelligen Organismen steht, dieselben Grundelemente wieder wie in dem einzelligen Infusionsthierchen. . . .

"Wieder und wieder der Beweis von dem göttlich einfachen aber auch göttlich gewaltigen Gesetze der *Einheit der Natur*" (p. 18).

The entoderm of Dicyema illustrates one or two points of interest in this connection. We have here an organ in which, as often happens, in parasitic degradation, cell-formation has been dispensed with. The entoderm remains throughout life as a single cell, and the whole process of reproduction, for both kinds of embryos, is carried on *in the body of this cell* without any cellular organs whatever.

In one respect this unicellular organ, which was undoubtedly once multicellular,

What is the difference between an organization embracing one cell and one embracing two or many cells? Certainly the essential difference cannot lie in the *number* of cells. We must look entirely behind the cellular structure for the basis of organization. Even a highly differentiated organism may reach a relatively late stage of development just as well without cell-boundaries as with them, as we see so well illustrated in the insect egg. If we fall back on the number of nuclei as the essential thing, then we shall have to reckon with multinucleate infusoria. In these forms do we not see that it is always the *same* organism before us, as we follow its history through the whole cycle of nuclear phases?

The essence of organization can no more lie in the number of nuclei than in the number of cells. The structure which we see in a cell-mosaic is something superadded to organization, not itself the foundation of organization. Comparative embryology reminds us at every turn that the organism dominates cell-formation, using for the same purpose one, several, or many cells, massing its material and directing its movements, and shaping its organs, as if cells did not exist, or as if they existed only in complete subordination to its will, if I may so speak.

In the phenomena of regeneration and embryogenesis we find abundant evidence. For the present I must limit myself to a few features of development.

Perhaps the peculiar formation of the embryo Toad-fish (Batrachus) is as instructive a case as I am acquainted with.

is quite unique, for it may become the receptacle of nuclei belonging originally to other cells; in other words, *it becomes multinucleate, not by the multiplication of its own nucleus, but by the acquisition of exotic nuclei.*

The acquired nuclei are what I have called elsewhere the "residual" nuclei, which are left over when the formation of "infusoriform embryos" ceases. Each of these nuclei enters into vital relations with the cell, *and each undergoes the differentiations characteristic of the true entoderm nucleus*, so that in the end they can only be distinguished by their positions. This seems to show that the differentiation of nuclei may be controlled by the cell to which they are transplanted.

One of Boveri's observations* shows that the same may be said of the chromosomes. One or more of the chromosomes, normally eliminated in the polar globules, are sometimes carried into the cleavage-nucleus. The supernumerary chromosomes here undergo the regular transformations, quite unlike those which they show when carried out in the polar globule.

* Zellen-Studien. Heft 2, pp. 171–175.

If one will take the trouble to compare this formation with the ordinary type of teleostean development, he will not fail to see that the organizing forces, whatever they may be, operate to form an embryo under peculiar difficulties. It will be seen towards the end of embryogenesis that the material of the germ-ring, owing to the enormous size of the egg, has to travel over quite a long distance, in order to reach the embryo. A very thin bridge of cells connects the hind end of the embryo with the closing germ-ring, and this bridge is formed by the migrating cells of the germ-ring. What determines this wholly exceptional movement of the cell-material required to form the embryo? Is it possible that the cells move as so many independent individualities? But they do move, and no doubt in obedience to directing influences, acting, not in the cells as individuals, but in and through the entire formative material, irrespective of cells.

Whoever doubts this would do well to study more faithfully the *living* embryo during its formation. If the cell-ghost should still haunt his vision, I would suggest still another field for study. I would suggest first of all that he try to get as clear a notion as possible of the formation of the archenteron in Amphioxus, Petromzyon, and the Frog. The case of the reptile might then be studied with profit. Next the "chorda-canal" of mammals, and finally Kupffer's vesicle in the teleost.

There is no longer any doubt in my mind — and here I am in accord with most authorities on this subject — that this little vesicle is a reminiscence of the archenteron. The development of Gecco, as traced by Ludwig Will, removes, as I think, the last doubt on this point.

If the development of Kupffer's vesicle be studied in the light of its phylogenetic significance, and studied in the *living* as well as the dead egg, I cannot help thinking that candid reflection on the facts will be sufficient to force conviction to the standpoint here taken.

Having learned the meaning of the vesicle, one should trace step by step its mode of origin in the pelagic fish-egg. Here one may see this remnant of an archenteric cavity arise, not

inside the embryonic tissues as in the eggs of fresh-water fishes, but actually outside the tissues on the inner face of the embryo, near its posterior end. Its whole ventral and lateral boundary is formed, not by archenteric cells, but by a periblastic layer often as thin as the wall of a soap-bubble, and completely free from all nuclei. It does not even arise as a single cavity, but as numerous minute cavities that look like a cluster of granules. These expand, flow together gradually, and finally form one bubble-like vesicle projecting almost wholly into the transparent yolk. Having attained a maximum size, its slightly concave roof becomes more and more deeply hollowed out, and thus it comes to inclose more and more the cavity, while the latter gradually shrinks in size, and finally vanishes as the true cell-walls close up. Such is briefly the history of this floor-less form-reminiscence of what is a more substantial rudiment in many other embryos.

This remarkable reproduction of a form-phase that is to last only for a few hours and then pass away without leaving a visible trace of its existence, cannot be explained as due to cell-formation nor as the result of *individual* action or interaction on the part of the cells. The embryonic mass acts rather as a *unit*, tending always to assume the form peculiar to the state of development reached by its "essential architectonic elements" (Brücke) — elements that are no less real because, like the atom and molecule, they are too minute to be seen by the aid of our present microscopes.

That cells as such do not participate in this formative act, is shown by the mode of development of the vesicle and by the absence of cells in its ventral and lateral walls. This fact, the absence of cells, has actually been urged recently against the identity of the structure with Kupffer's vesicle, — an error which one is likely to fall into only while under the delusion that acellular walls cannot be homologous with cellular walls.

The evidence furnished by Kupffer's vesicle will doubtless lose much of its force with those who have not had an opportunity to study the subject sufficiently to form an independent opinion about it. To some who are better acquainted with the structure, its meaning may still appear to be somewhat prob-

lematical, and the evidence drawn from it as therefore unsatisfactory. It would be useless in such a case to urge the point, and also wholly needless, as examples abound that are not open to such objections.

The form-changes by which the fish blastodisc passes into the germ-ring stage are examples of this kind. It is well known that the transformation of the blastodisc just before the appearance of the germ-ring is quite rapid, at least in the pelagic fish-egg, and also *quite independent of cell-formation.* The discoidal germ-mass suddenly thins out, but not uniformly in all parts. The half of the disc in which the embryo is to be formed remains thick, anticipating as it were the axial concentration which is to follow, while the half lying in front of this is rapidly reduced to a thin epithelial membrane. This *regional* differentiation of the outer layer and the concomitant formation of the germ-ring, including the forward movement of the embryonic plate ("head process"), which advances in an axial direction to the very centre of the disc, are indubitably accomplished, not by the aid of cell-formation, but by formative processes of an unknown nature, but nevertheless real and all-controlling. Cell-formation, to be sure, goes on, but it seems to me certain that it has no *directive* influence on the formative processes. The cleavage runs on from beginning to end, regularly or irregularly, without modifying in any essential way the form of the blastodisc. All at once, when this segmentation has been carried to a certain point, the transformation sets in and goes rapidly on, without interrupting cell-formation, but to all appearance quite independently of it.

In the axial concentration of the very broad embryonic plate we see a formative process that can have nothing whatever to do with cell-division. Again, in the establishment of the caudal end of the embryo, long before that part of the germ-ring which represents, historically at least, this end can be brought into place, we have another decisive test of formative power asserting itself, not only independently of cell-division, but also against all the obstructions interposed by the yolk. This prepotency of the "plastic power" (Schwann) is seen to great

advantage in the pelagic fish-egg, but still better in the Toad-fish-egg. It is needless to cite further examples of this sort, for the embryology of every animal is full of them, and no one can fail to find who looks for them.

If the formative processes cannot be referred to cell-division, to what can they be referred? To cellular interaction? That would only be offering a misleading name for what we cannot explain; and such an answer is not simply worthless, but positively mischievous, if it put us on the wrong track. Loeb's experiments in heterogenesis furnish a refutation of the interaction theory. The answer to our question may be difficult to find, but we may be quite certain that when found it will recognize the regenerative and formative power as one and the same thing throughout the organic world. It will find, as Wiesner has so well insisted, a common basis for every grade of organization, and it will abolish those fictitious distinctions we are accustomed to make between the formative processes of the unicellular and multicellular organisms. It will find the secret of organization, growth, development, not in cell-formation, but in those ultimate elements of living matter, for which *idiosomes* seems to me an appropriate name.

What these idiosomes are, and how they determine organization, form, and differentiation, is the problem of problems on which we must wait for more light. All growth, assimilation, reproduction, and regeneration may be supposed to have their seat in these fundamental elements. They make up all living matter, are the bearers of heredity, and the real builders of the organism. Their action and control are not limited by cell-boundaries. As Heitzmann and others have long insisted, the continuity of these elements is not broken by cell-walls. The organization of the egg is carried forward to the adult as an unbroken physiological unity, or individuality, through all modifications and transformations. The remarkable inversions of embryonic material in many eggs, all of which are *orderly* arranged in advance of cleavage,[1] and the interesting pressure experiments of Driesch by which a new distribution of nuclei is *forced* upon the egg, without any sensible modification of the

[1] As will be shown later.

embryo, furnish, as I believe, decisive proof of a definite organization in the egg, prior to any cell-formation. The opinion expressed by Huxley in his review of "The Cell-Theory," in 1853, forms a fitting conclusion to this introductory sketch.

"They [the cells] are no more the producers of the vital phenomena than the shells scattered along the sea-beach are the instruments by which the gravitative force of the moon acts upon the ocean. Like these, the cells mark only where the vital tides have been, and how they have acted."[1]

[1] British and Foreign Medico-chirurgical Review, Vol. XII, p. 314. Oct., 1853.

SEVENTH LECTURE.

BDELLOSTOMA DOMBEYI, LAC.

A STUDY FROM THE HOPKINS MARINE LABORATORY.

Problems in Biology are inexhaustible, they renew themselves continually. They appear with kaleidoscopic changes to successive generations of men, who receive them to study in new light and from new standpoints. The results of these studies are not all as stable as the problems themselves. These results present themselves to us as facts, and as theories based on facts. Although one well-established fact is worth much speculation about its significance, yet, without the aid of well-considered theories concerning the causes which lie back of the facts and the meaning of the facts in themselves, progress were well-nigh impossible.

These are trite sayings, but they have very appropriate applications to the problems of the nature and functions of the ear at the present time. The general problems of the morphology and physiology of the vertebrate ear are much the same as they were when first taken up, for the two questions, "How is the vertebrate ear constructed?" and "How does it operate?" still express the aims of our researches in this field as well as they did when man first began to study into his auditory anatomy. But our conception of the manner in which these problems are to be solved at the present day is no longer what it was fifty years ago, or, indeed, ten years ago. We now appreciate the full force of the requirements which the present condition of morphological and physiological science demands in the investigation of these problems. To know the structure and function of our own ear, we find it not only necessary to study the adult anatomy and function of the ear in man, but also in *every other* vertebrate form as far as pos-

sible. Nor can we stop here. We must trace out the *ontogeny* of this organ in all those forms whose adult anatomy we study, and in so doing apply the known laws of physics and of chemistry to the development of their forms and contents. We must trace out the phylogenetic development of the ear by the comparative study of what we have gained from the above outlined investigations, combined with knowledge from two sources yet untouched, viz., the morphology and physiology of those sense-organs, genetically related to the ear sense-organs, and the scanty store of facts pertaining to our problems which palaeontology can give us. Investigations undertaken on a basis less broad than this, or which do not fall within some one of the categories above mentioned, are sure to fall far short of the solution in the first case, and, in the second case, to be of little or no service in the general progress toward the final solution of these problems.

It is thus made apparent, I think, that although the physiological problem is not as extensive as the morphological, it is not for that reason less difficult or more likely of an early solution, for its final solution depends quite as much upon our advances in a knowledge of the anatomy and physiology of the central nervous system as upon experiments upon the ear itself. With this prospect of long-postponed final solution, facts which have direct bearing upon any of the fundamental topics of ear physiology, are all the more valuable. One such fact I wish to announce, viz., that there is at least one vertebrate which does not depend upon its internal ears for the equilibration of its body. I had previously concluded that the semi-circular canals were not organs of equilibration, and incidentally during my anatomical work I was able to determine that the ear of this vertebrate is in no way more concerned in maintaining the position of its body in space than are the other sense organs.

In the lectures for 1891, I brought to your attention certain facts and arguments to illustrate the gradations of structure of the ear found among vertebrate animals, which have led up to the production of the human ear. And I showed the manner in which the ear of Marsipobranch fishes presents us with

the simplest form of this organ now in existence. In continuance of my investigations of this important organ, it became necessary for me to study in the living condition some representative of this group of fishes, of which we have only two species in American waters, viz., the Myxine, or Hag-fish, of our Atlantic coast, which is found from Greenland to Cape Cod, and the Bdellostoma of the Pacific coast, which has been found along nearly the whole Pacific coast-line of both North and South America. In reply to a letter of inquiry addressed to President Jordan, of Leland Stanford Jr. University, who is, as you know, preëminent for his knowledge of American Ichthyology, I learned that Bdellostoma occurs in great abundance in the Bay of Monterey and that it could be obtained there with comparative ease. These two factors determined the direction of my journey, and I accepted the kind invitation extended me by Profs. C. H. Gilbert and O. S. Jenkins of Stanford University, placing the facilities of the Hopkins Seaside Laboratory at my disposal. This marine station is an adjunct of Stanford University and has just passed through its second season.

It is a great pleasure for me to be able to tell you of the true scientific spirit in which the affairs of this rapidly growing laboratory are conducted, and to acknowledge the generous assistance accorded me by the directors, Drs. Gilbert and Jenkins. In the interest of biology in America, it should be made widely known that Mr. Timothy Hopkins of San Francisco, in whose honor the laboratory has been named, has alone rendered it possible to found the institution, and he has provided it with the means of growth. With an insight into both the great scientific and practical importance of the biological researches rendered possible by such a station, an insight as rare in a man of affairs as it is admirable, Mr. Hopkins has supplied the indispensible funds for the undertaking, and has assured the directors of his desire to have the station grow, not by words alone, but by meeting the expenses of the additions to the station buildings and equipment. In doing this Mr. Hopkins has not been unmindful of the library which is a fundamental need of all research work, for he proposes to make and keep it the most complete collection of biological

literature in connection with any biological laboratory in this country. From this time on the station is to be kept in readiness for the use of investigators at all seasons of the year.

Thus you see the needs of biological research at the seashore are appreciated and provided for on the Pacific coast in a manner worthy of emulation by men of means on this side of the continent. How long must we wait for the needed financial support of our permanent station here? Our needs are urgent, and they should be met at once. Here is a great opportunity which will hardly recur in this generation.

The Hopkins Seaside Laboratory stands near the beach on a rocky point forming part of the peninsula which constitutes the southern boundary of the granite basin known as the Bay of Monterey. It is in the town of Pacific Grove, two miles distant, west from Monterey, and 128 miles south of San Francisco. The station consists, at present, of a plain frame-building, very similar to the original building of the Wood's Holl Laboratory, though additions to it are to be made this year. A pump and tank house is added to the east end of the building for supplying the station with sea-water, which is pumped up more than twenty-five feet above the sea level to the supply tank, from which it flows through the various aquaria in the building, and thence into the large, out-of-door ground aquarium, in which large animals and class supplies of the hardier small animals are kept. The fauna and flora of the Bay of Monterey are very rich; but, until they are more thoroughly studied, we cannot accurately estimate the certainly very large number of forms, both rare and common, or the abundance of the individuals of the species most valuable for the work of the station.

In general, we may say that the fauna and flora is subtropical, and it is rendered so by a very constant, almost unvarying temperature, which averages 65° the year through, and also by its nearness to the northern bounds of the tropical seas of Central and South America.

One of the features of the station is its nearness to a Chinese fishing village, from which much of the material for work is obtained. Fish are caught almost entirely by means

of trawl lines; and, while nets of several kinds are used from boats, no pound nets, such as we find in the shallow waters about here, are used, because of the depth of water and the rocky character of the coast.

On arriving at the station, it became at once apparent that I should have to depend upon the Chinese fishermen for the collection of my material. Bdellostoma is so abundant as to be pestiferous to the fishermen by clogging the lines with their peculiar tenacious *slime*.

Bdellostoma, as you know, belongs to the so-called Myxinoid fishes, this name having been applied to them on account of their unusually slimy bodies. The first of these fishes to be discovered was the European Hagfish, which is identical with our Atlantic coast species, and it has been classed with the Worms, Mollusks, Amphibia, and finally with the Fishes, where it properly belongs. The great Linnæus called it a worm, even though his attention was called to its affinity with the fishes. Bdellostoma lives on the bottom of the ocean, out to the depth of one hundred fathoms and more, but seems occasionally to ascend fresh-water streams for short distances. It is supposed to be in its habits more or less parasitic on the Halibut, agreeing in this respect with Myxine, which is parasitic on the Codfish. I have seen large skins of the Halibut beautifully deprived of all contents save the skeleton, and with but a single opening in the region of the gills to show where the devastator had entered the body. Bdellostoma is about twenty inches in length (an average size), but varies from about fifteen inches to twenty-five inches in length. The relative proportions of the body in the two sexes, as well as in youth and age, are much the same. The body is cylindrical in shape, but flattened from side to side in the tail region. In color it varies from light pink to a dark-purple brown. The color has a pinkish tinge during life, owing to the shining through of the red blood in the vessels of the white skin below the surface coloration spoken of above. The median ventral line remains uncolored from the region of the yolk duct or umbilicus to the cloaca, and in old individuals the snout and a broad stripe on the ventral surface of the body

is white. In other words, as age increases, those parts of the body which come into frequent contact with objects other than water lose their purple color. The color grows gradually darker from the ventral surface to the dorsum. The skin is attached to the surface of the body of the animal by a thin median dorsal ligament and by a broad median ventral area of trabeculous processes. The gill holes show a narrow, whitish border, which is especially well marked about the ductus œsophago-cutaneus, or the tube which permits the water to escape from the œsophagus without going through the gills (Fig. 9, *D*). The cloacal opening is edged with white. Over the region of the eyes the skin is pigmentless and transparent, so that light penetrates freely to the eye below; but this corneal membrane is not in any way invaginated or fixed to the surface of the eye, and, since the skin in this region is very movable, the transparent area is very much enlarged when compared with the size of the eyes (Figs. 1 and 5). The latter are small and devoid of pigment in the retina, as far as can be discerned from the exterior, and the whole bulb is imbedded in a fat pad between the two diverging cutaneous branches of the V nerve where they issue from the sides of the head. The fish are very sensitive to light, and seek for covering until the head is shaded, when they come to rest, it may be, with most of the body exposed.

Bdellostoma is not provided with appendages, *i.e.*, pectoral or pelvic fins, and depends for locomotion upon movements of its body, eel-fashion. It is a graceful and rapid swimmer. In the aquaria it remains most of the time quiet upon the bottom, resting upon its ventral edge or side. In order to maintain an upright position, *i.e.*, back uppermost, it is necessary for it to curve the body more or less, in order to provide a base of support. Oftentimes the curve is slight, as is always the case when the animal is in poor health. The normal position is that of a right or left-handed coil; and they coil both ways, apparently to rest the muscles occasionally (Fig. 3, *a* to *h*). Sometimes the whole body is taken up in the formation of the coil; again, often only the posterior part is used, thus the head-end being reserved free, and held straight or bent into an

S-shaped figure. A slight disturbance or an internal irritation will cause them to uncoil slightly, when, like a delicate watch-spring, they recoil and vibrate for an instant before coming back to rest (Fig. 3, *a* and *b*). This motion is one of the most beautiful illustrations of muscular elasticity which I have ever seen. As shown in the Fig. 3, Bdellostoma can curve its body into very complicated figures, the most complex being the double and triple knots into which the fish can quickly tie itself for the purpose of removing anything attached to the surface of its body. This it accomplishes by keeping its body in motion through the knot, and tying up as fast as it comes untied. When in a position of complete rest, the fish rests equally well with only a slight bend in the head or the tail region, though it is the tail that is normally used as a support for the body to lean upon. When irritated the fish discharges from a series of minute holes in the skin, running along either

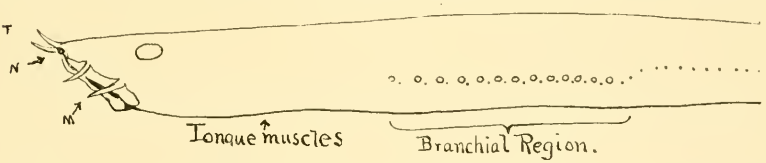


FIG. 1. — The Cephalo-Branchial region of Bdellostoma dombeyi seen from the left side to show the truncated anterior end of the body and its complement of feelers. The position of the eye-spot and the mutual relations of the external branchial pores and the thread gland pores is also given, ¼ natural size.

side of the body, which project like minute nipples above the surface of the skin, a milky-white fluid, which almost instantly disappears from sight when the fish is in the water. These holes are the openings into the so-called mucous sacs or, as I shall designate them hereafter, the *thread cell pockets* or *nidamental organs*. These sacs are imbedded in the muscles of the body, and not in the subcutaneous tissue, as Jackson affirms in his recent edition of *Rolleston*. The discharge is in the form of minute jets, and is caused for the most part by the muscular contraction of the skin about the body. The fluid is composed of minute bodies about the size and having something of the appearance of starch grains. Each grain is a *thread cell*, and was originally a columnar cell, derived from

the skin lining the pocket, and which has been transformed into one continuous thread in such a manner that when brought into contact with water it unrolls itself in layers, much like a spool of knitting-silk, when it comes to pieces in layers, and straightens out in the water in an incredibly short space of time. After such a discharge, if one attempt to take the fish out of the water, one will notice that the fish is pushed away from before the hand, and that it is impossible to touch it ; and, further, that there is an invisible something between one's hand and the fish. By pouring off the water, or raising the fish above the surface, you find the creature inclosed in a transparent, gelatinous mass of about the consistency of thick egg albumen, which is extremely slippery, and yet adhesive and, at the same time, not readily broken into pieces, notwithstanding its apparent gelatinous nature. A single fish will quickly fill a bucket with this so-called slime ; *i.e.*, convert the water into such a thick jelly. But the amount of solid substance, *i.e.*, the mass of the exploded thread cells required to hold the water together in this way, is not equal to a piece of tissue paper the size of the bottom of the bucket.

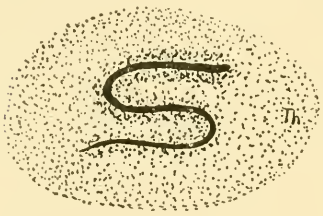


FIG. 2. — To illustrate the way in which Bdellostoma surrounds itself with a mass of tangled threads, in the meshes of which a great quantity of water is held. The consistency of the mass is that of egg albumen.

You see from this short account that Bdellostoma is provided with a simple but effective means of protection from its enemies, and at the same time with a good nest-building apparatus, for it can at a moment's notice secrete a dwelling-place exactly fitted to its body without stirring out of its place, no matter what position it may be in. Oftentimes these nest-shaped masses are brought up with the fish from the bottom. Bdellostoma is not so much of a parasite as it is commonly reputed to be. It lives on or close to the bottom of the sea, and like its relative, Myxine, it seems to prefer mud-covered surfaces in which it may partly conceal itself. It feeds upon fishes of the Teleost group. It is not known that they ever

disturb Elasmobranchs. They devour their own eggs, and I have frequently taken the remains of small squids from the stomachs of these animals. It is certain that they do penetrate the bodies of such fish as the Halibut, the Flounder, the Rock Cod and Codfish, though there is not the slightest evidence that they do so while the fish is in the living condition. They might readily do so, however, and the fishermen all believe that they do attack living fishes and bore their way into them. This much is certain ; very frequently fish are taken in the trawl nets from which one may see the Bdellostoma issuing in haste to get into the water again. These fish hulks are found to be, usually, thoroughly despoiled of all their soft parts. They retain the fish form by means of the skeleton within an intact skin — intact with the exception of the small hole in the region of the gills through which the squirming fish was seen to come. Since Bdellostoma bites the sardine-baited, trawl-line hooks of the fishermen with extreme avidity,[1] and is able to swallow pieces of fish or other food nearly as large as its own ordinary diameter, it does not seem to be entirely correct to designate it a strictly parasitic animal. And if it is not parasitic, its sense organs can hardly be said to have been degraded by parasitism. If they are not degraded, they must represent primitive conditions, and so far as the structure of the nose, eye and ear are known to us, they contain absolutely no anatomical characters which would justify the conclusion that they are degraded from a more perfect ancestral condition. The eye lies beneath the skin, it is true, but this is doubtless a stage in the phylogenetic development of the eye, just as the existing and very primitive condition of the nose is, without question, a stage in the genesis of the nose of higher forms.

The head end of Bdellostoma is obliquely cut off from above, downwards and backwards, in such a fashion as to bring the anterior end of the long tubular nose at the extreme front end of the body ; and since this anterior nasal aperture is

[1] The trawl-lines are often brought up with as many Bdellostomas as other fishes, and my Chinaman assured me that sometimes nearly *all of the hooks* were taken by this Hagfish ; or, as he expressed it, "Evely hook — one Sliklostome," *i.e.* Cyclostome — he having learned the scientific name of the creature from the students of the Hopkins Laboratory.

surrounded by four elongate conical feelers, and is further under the control of a set of muscles which effect its movements in all directions about the long axis of the body, we see that Bdellostoma is provided with a very sensitive and effective organ of touch, and as such it makes continual use of it. Each of the feelers is erectile ; that is to say, it may be laid back against the side of the head or thrust out in any given direction at the will of the creature (Fig. 4). Each feeler is richly supplied with nerves and blood vessels, and has a slender and flexible, though very tough filament running through it to give it strength and to help in keeping its form. These

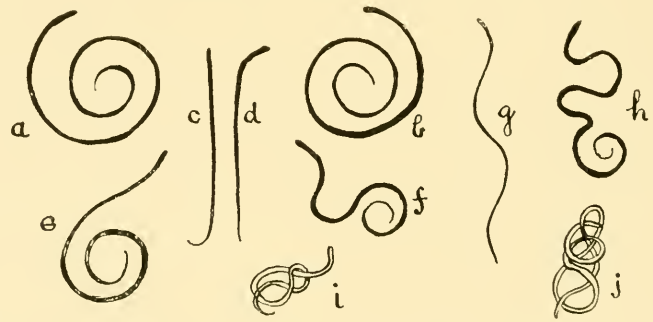


FIG. 3.— Ten representations of the positions taken by Bdellostoma at rest (*a*, *b*, *c*, *d*, *e*, *f*,) and in motion (*g*, *h*, *i*, *j*). *a* and *b*, right- and left-handed coils. *c*, with bent tail to act as support for body. *i* and *j*, single and double bow-knots, into which Bdellostoma ties itself in order to draw its body through the knot for the purpose of removing the thread cells or other foreign substances from the surface of the body.

sensitive feelers about the nostril give warning of the presence of solid particles in the water which is being respired, for a constant stream of water is drawn into the nose to pass into the mouth through the hole in the roof of the palate and thence onward to the gills. The genuine nasal nerve end-organ is the simplest in structure of any presented to us by the vertebrate series, and consist (Fig. 7) of seven semi-oval plates of mucous membrane which hang from the roof of the nasal cavity down into the stream of water as it turns downward to pass into the mouth. These plates are bilaterally disposed in sets of three, on either side of the median plate, which is

the largest of the seven. The others decrease in size from within outwards. The median plate is characterized by its larger size, and the possession of a terminal knob at its anterior end. This knob is continued forward for some distance along the roof of the nasal tube as a continually decreasing ridge. So far as my investigations have gone, the olfactory nerves end in Bdellostoma, as they do in Petromyzon, on either side of this median raphe, so that we now know that all Craniate vertebrates possess bilaterally symmetrical special sense organs since neither group of the Cyclostome forms an exception to the rule.

The eyes in Bdellostoma have been considered greatly degenerated, and this view is still generally accepted on the strength of Johannes Müller's view of their condition. I feel assured, from a preliminary examination of the eyes,—and my examination is more extended than the one upon which Müller based his conclusion,[1] — that the Bdellostoma eye really represents a phylogenetic stage in the differentiation of the vertebrate eye. Two striking features of this eye are the lack of visible pigment in the retina when viewed from the outside, and the absence of a genuine cornea. From a study of the adult condition, it is highly probable that the skin of the body is never drawn into the formation of the eye as we meet with it in the fishes ; consequently, neither crystalline lens nor cornea are present. The original optic cup budded out from the growing brain wall forms all there is of this creature's eyeball, and hence it stands as an important and extremely interesting intermediate stage between the Branchiostoma condition and that obtaining among the rest of the sub-kingdom. The large transparent oval spot over each eye serves as a cornea, permitting the light to enter the eye no matter how much the skin may be moved out of its ordinary position. The eyes, along with the rest of the body beneath the skin, are constantly bathed in the lymph which occupies the space between the skin and the muscles.

[1] Kohl (Zoolog. Beiträge) has given us an account of the histology of the eye in Myxine glutinosa and he adheres still to the old view of the degeneracy of this organ.

The ear of Bdellostoma lies imbedded in the cartilage of the base of the skull and below a thick layer of muscle. The membranous portion is essentially like the ear of Myxine, and the reader is referred to the previous volume of lectures where I have described the Hagfish's ear in considerable detail.

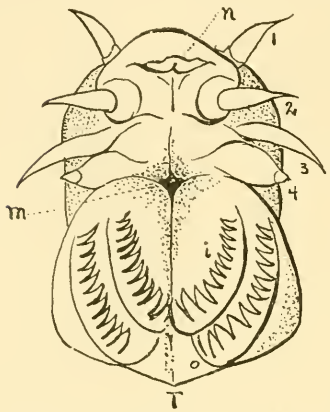


FIG. 4. — A full front view of the face of Bdellostoma. *n*, nasal aperture. 1, 2, 3 and 4, the tentacles or feelers. *i* and *o*, the inside and outside rows of teeth. *m*, the mouth. *T*, the tongue, which is here thrust forwards out of the mouth and nearly conceals the opening. The tongue stands here at right angles, in two directions, to its usual position inside of the head. nat. size.

Bdellostoma lacks all traces of the system of lateral line organs so far as is yet known, and it has no representatives of the other specialized sense organs of the skin, common to most fishes.

Isolated sensory cells are the only special sensory structures yet found in this animal's skin. The absence of these surface sense organs, together with the entire lack of appendages, renders Bdellostoma an extremely valuable animal with which to perform ear-function experiments; this is due in great part, of course, to the very simple condition of the ear itself.

Upon cutting through the scaleless and relatively thin skin along the ventral line, the body muscles come to view. Of these, there are two principal layers covering the whole body. When they are cut through along the ventral line, from the mouth to the cloaca, most of the viscera are exposed. In the branchial region, as shown in Fig. 9, we find the heart with its large ventral aorta running forwards, giving off gill-arteries as it goes, in such a manner that either side receives as many branches as there are gills on that side. The gills themselves are compressed sacks, circular in outline, which assume a sub-globular shape when they fill with water. The current of water which we have traced from the nose into the mouth, passes back through the œsophagus, and then out into the gills by as many short tubes leading from the œsophagus

as there are gills. After entering these sacks the water is forced out through the sides of the body through other short tubes which connect the gill-sacks with the surface of the skin. Here they form the circular gill-holes to be seen on the side of the head region of the animal. Two features in the arrangement of the gills deserve attention. First, the large tongue-muscle seen in Figs. 5, 9 extends backwards among the gills, in this individual separating the anterior five pairs, effectually preventing any contact between the gill-sacks of the opposite sides and also bringing about a compression of the sacks in this

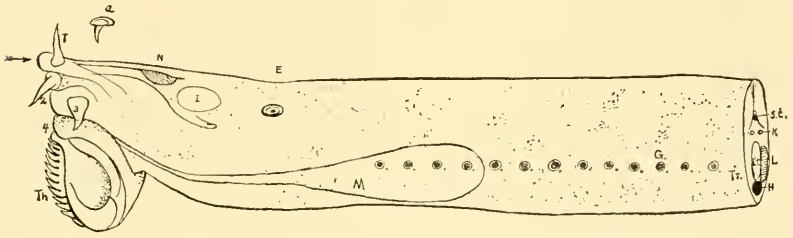


FIG. 5. — A side view of the cephalo-branchial region of Bdellostoma. Some of the internal organs are drawn into the figure, the body walls being thought transparent. The arrow shows the direction of the respiratory current of water toward the nose. *T* 1, 2, 3, and 4, the tentacles. *Th*, the teeth, which here are erected and thrown outwards by the unfolding of the tongue. *N*, olfactory organ. *E*, the ear. *I*, the eye. *M*, the club-shaped tongue-muscle. *G*, the external branchial pores. *Tr*, the thread gland pores. *S. C.*, the spinal cord. *K*, the kidneys. *It*, the intestine. *L*, liver. *H*, heart. Below and behind each gill opening is seen the opening of a thread gland. *a*, above the figure, the single, median, pharyngeal tooth.

region whenever the tongue-muscle is in action ; second, the apparent relation of the point of bifurcation of the branchial aorta to the posterior end of this tongue-muscle. The number of gills varies to an astonishing degree, and the significance of this variation has not heretofore been the subject of any serious inquiry so far as I am aware. There are two series of facts concerning this flexibility of the branchial system which deserve separate consideration. First, the number of gills of individuals from *different localities* varies from 6 on either side to 14 on either side, with the observed intermediate stages as follows:

Six gills on both sides, 6 on one, 7 on the other side, and 7 gills on both sides. This series is from the Cape of Good Hope, *i. e.*, practically Indian Ocean, and was discovered by Johannes Müller in 1834. Müller originally gave a separate specific name to each of these varieties but later concluded that they formed only a single species. No representatives of the series with more than 7 gills on both sides and less than 10 on both sides, have as yet been recorded, or so far as I know, observed. This leaves a gap of 2 gills. But from 10 gills on both sides, up to 14 gills on both sides the series is complete and is as follows: 10 gills on both sides, 11 gills on both sides, 11 gills on one side, 12 on the other, 12 gills on both sides, 12 on one side, 13 on the other, 13 gills on both sides and 14 gills on both sides. It is interesting to note the fact as first made out by Willey that during its larval development, the number of primary gills laid down in Branchiostoma[1] varies from about 8 to 16, with an average number of 14. This may be a mere coincidence, but I am disposed to look upon this as a fact of fundamental importance. Kowalewsky first studied the growth of the gills in Branchiostoma, and Willey has greatly increased our knowledge of the processes of differentiation met with in these organs. Unlike other members of the vertebrate stock this animal has the gills on one side laid down before those of the opposite side make their appearance, but this is a mere retardation and not an essential difference. The second gill-slit is the first to appear and is soon followed by the first, after which increase in number takes place, only from before back-

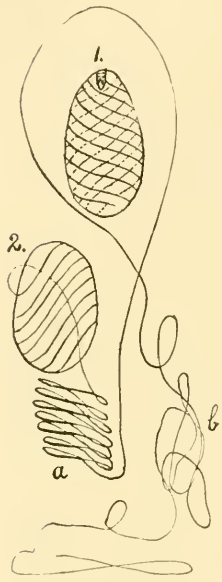


FIG. 6.—Two thread-cells. 1, unexploded, showing two crossing layers of the threads. 2, partly exploded and unravelled thread-cell. *a*, a portion of a coil which has slipped off the cell. *b*, the unravelled thread, much magnified.

[1] It is advisable on account of priority to use the term Branchiostoma in place of the more familiar term Amphioxus, as Prof. E. A. Andrews has recently pointed out in his paper on *An Undescribed Acraniate*. Studies Biol. Lab. Johns Hopkins Univ., V, no. 4, 1893.

wards. These simple primitive slits increase in number until about 14 of them are laid down, when a stage is reached during which no more slits are formed in the antero-posterior series. This stage has been designated the critical stage by Willey. When the activities of growth again manifest themselves in these organs it is an entirely new fashion and results in the splitting of each primitive gill-slit into two by the downward growth of a *median gill-bar* which thus separates an anterior from a posterior portion of the original aperture. After this process has begun, increase in the number of gills from before backwards again takes place; but is now evidently related to the acquired habits of the animal, as I have already pointed out in another place.[1] In the matter of gills, we see that Bdellostoma approaches very close to Branchiostoma, and in this sense the Myxinoid retains very primitive relations of its gill-apparatus. To return to the question of the variability of the gills in Bdellostoma, what can be its significance? Have we to do with an increase or decrease in the number of gills of this animal, seeing that by far the largest number of individuals now existing have either 10 or 12 gills on both sides of the body? I think there can be no doubt as to the answer. We are here dealing with a reduction in the number of gills and in no case with an increase. This conclusion is supported by the fact that the more highly differentiated Marsipobranchs have a smaller number of gills, and this is true not only of Myxine, which belongs to the same group as Bdellostoma, but in a greater degree of the Petromyzonts. In view of these facts it is extremely probable that the ancestors of Bdellostoma were provided with about 13 to 14 gills, and this number may be taken as representing the numerical relations of the branchial apparatus of the primitive forms of the vertebrate stock generally. The relation of the base of the tongue-muscle to the gills is of interest, and here again we find great variability. Müller found it to lie entirely in front of the gills in the 6 and 7-gilled forms from the Cape of Good Hope, and this condition obtains in Myxine so far as known. In Bdellostoma with 10 or 11 gills the base of this muscle may lie between the 6th and

[1] Concerning Vertebrate Cephalogenesis. — *Journal of Morphology*, IV, 1890.

8th pairs of gills, according to Putnam. In the 12 and 13-gilled forms I have found it between the 5th, or at most, the 6th pairs of gill-sacks.

In the material which I was able to collect at Monterey the following proportions of the several variations prevailed :

101	individuals	had	11	gills	on	both sides.
26	"	"	11	"	"	one side
		and	12	"	"	the other side.
208	"	had	12	"	"	both sides.
11	"	"	12	"	"	one side
		and	13	"	"	the other side.
8	"	had	13	"	"	both sides.
354	total number of individuals counted.					

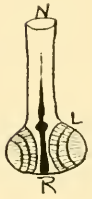


FIG. 7. — The olfactory organ seen from its ventral surface. *N*, nasal tube. *L*, the nasal plates, of which there are three on either side of the median raphe. *R*. × 2 diameters.

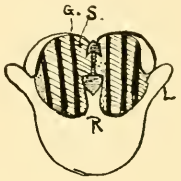


FIG. 8. — A transverse section of the olfactory organ of Bdellostoma, showing the nasal plates cut across *G*, and also partly in surface view, *S*. *R*, the raphe, and *L*, the lateral wall of the nasal capsule. × 4 diameters.

Of the eight 11–12 variation, where the position of the gills was noted, four had 11 gills on the right side and 12 on the left, while the other four were just the reverse, with 12 gills on the right side and 11 on the left. The same alternate variation holds true of the 12–13 variation. This fact of alternate variation proves conclusively that the variability in number of gills between the two sides is in no way connected with the formation of the ductus œsophago-cutaneus, which is always upon the left side.

The vascular system of Bdellostoma is being studied in Prof. Gilbert's laboratory at Stanford University, and I need not enter into the interesting relations which this system of organs sustains to the general variability of this animal,

especially with respect to the branchial arteries, whose position is shown in the cut. It is through the large median vessel A, that the blood is carried to the walls of the gill sacks, by means of the individual branches *a*, one of which leaves the main trunk, so long as it remains unpaired, for each gill.

Owing to the possibility of its playing an important part in the development of the variability of the gills, it is desirable to examine the nature of the so-called tongue and its muscles a little more in detail than we have yet done. In Figs. 9 and 5 these structures are shown as they appear upon slitting open the body muscles. The tongue forms a somewhat heart-shaped plate, when spread out flat as in Fig. 4, and is composed of two symmetrical lateral pieces. These halves each bear two rows of teeth, and during life, while in the mouth, they are inclined at an angle to each other, thus forming a trough, whose shape is maintained by the sides of the mouth. When, during life, the tongue is thrust out of the body, it is flattened out upon the anterior edge of the solid floor of the mouth cavity, as shown in Fig. 5, when it is in the best possible position for the use to which it is put, *viz.*, the rasping away of the flesh of the fish to which Bdellostoma has fastened itself; or, if it is a small bait, the rapid and certain forcing of the bait into the mouth cavity. The mechanism which effects this end is quite complicated, and I need not enter into a complete

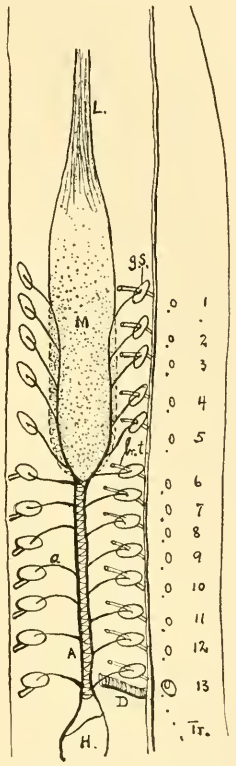


FIG. 9. — A ventral view of a dissection of the gills of a Bdellostoma dombeyi, with 12-13 gills, to show their relations to each other, to their blood supply, and to the surface of the body. *M*, the club-shaped tongue muscle. *L*, its tendon. *g. s.*, branchial sack. *1-13*, the external branchial pores or gill holes. *Tr.*, the pores of the thread glands. *A*, the ventral aorta. *a*, the branchial arteries. *D*, the ductus œsophago-cutaneus. *br. t.*, the internal branchial tube. *H*, the heart.

description of it here. It will suffice if I confine my remarks to the large club-shaped muscle which lies between the anterior pairs of gills. In cross section the muscle is seen to be composed of an outer thin ring — the section of the thin cylindrical muscle, and an inner solid muscular disc — the section of the solid, club-shaped muscle, which is made up of two equal lateral halves. Johannes Müller has called the outer one of these the hollow tongue muscle, and it certainly does at first sight seem to deserve the name.

The long tendon into which the tongue muscles taper, runs forward in a groove in the top of the cartilaginous floor of the mouth until it reaches the posterior border of the tongue, when it splits up into two unequal bundles of tendinous fibrillæ, which run forward and insert into the upper surface of the tongue body. These slender tendinous slips are so arranged that, when the tongue is drawn out of the mouth, the cartilaginous bars carrying the teeth are turned, so as to throw the teeth outwards and forwards in such a manner that their points are erected, and catch readily the instant the club-shaped muscle begins to draw it back into the mouth. The teeth are corneous structures,[1] borne upon the soft teeth papillæ, and the teeth of any one row are more or less fused together at their bases, so that when separated from their papillæ, they hang together in a row or plate. (Figs. 4 and 5.) Oftentimes, the inner tooth, which is also the larger, separates from the others, and the outermost tooth of the row is not always united with its fellow. Each tooth is an exceedingly sharp-pointed, conical body, flattened from above downwards, and curved from without inwards.

[1] I have made a special examination of the teeth of Bdellostoma dombeyi and Myxine glutinosa, and although I was desirous of finding the enamel cap described by Beard I was unable to do so. There is not the slightest trace of *bone* in any of the teeth of these two forms, and what Beard has taken to be such is doubtless much hardened horn, produced by the methods used by this author in preparing sections of teeth for microscopic examination. I have cut a large number of the teeth of Bdellostoma and of Myxine and have found no difficulty in sectioning them *in situ* when imbedded in celloidin. The Myxinoid teeth are cornefied sheaths of the epidermal elevations on the tongue plate, and in adult life show no trace of dentine or enamel.

The number of teeth in Bdellostoma varies as much, if not more, than the number of gills, so that the only two "*constant*" characters which Müller could find upon which to base a classification are both of them *extremely variable*. The limits of the variability of the teeth, so far as known, are as follows:—

Bdellostoma from the Cape of Good Hope, 6–7 gills, $\frac{8}{7}|\frac{8}{8}$, $\frac{11}{11}|\frac{11}{11}$, $\frac{12}{11}|\frac{12}{12}$.

Bdellostoma from coast of Chili, 10 gills, $1\frac{1}{7}|1\frac{1}{7}$ (Lacépède), $\frac{12}{11}|\frac{12}{11}$, $\frac{13}{12}|\frac{13}{12}$.

Bdellostoma from coast of California, 11–13 gills, $\frac{9}{9}|\frac{8}{9}$, $\frac{12}{11}|\frac{12}{11}$.

The California series which I have counted is the only one extensive enough to give opportunity for the observation of the variations, although Müller evidently had nearly extreme forms in the three individuals whose dental formulæ he recorded.

In 22 individuals with 11 gills I found the dental formulæ to be as follows:—$1\ \frac{10}{9}|\frac{9}{9}$, $1\ \frac{10}{10}|\frac{9}{10}$, $4\ \frac{10}{9}|\frac{10}{9}$, $1\ \frac{10}{9}|\frac{10}{10}$, $1\ \frac{10}{10}|\frac{10}{10}$, $1\ \frac{10}{10}|\frac{11}{9}$, $1\ \frac{10}{9}|\frac{11}{9}$, $1\ \frac{10}{10}|\frac{11}{10}$, $3\ \frac{11}{10}|\frac{10}{10}$, $1\ \frac{11}{11}|\frac{10}{10}$, $5\ \frac{11}{10}|\frac{11}{10}$, $1\ \frac{11}{11}|\frac{11}{10}$, $1\ \frac{12}{9}|\frac{11}{9}$, $2\ \frac{12}{11}|\frac{12}{11}$.

In 61 individuals with 12 gills the following dental formulæ occurred:—$1\ \frac{8}{9}|\frac{10}{10}$, $1\ \frac{9}{9}|\frac{8}{9}$, $8\ \frac{9}{9}|\frac{10}{9}$, $1\ \frac{9}{10}|\frac{10}{9}$, $1\ \frac{9}{10}|\frac{10}{10}$, $2\ \frac{10}{9}|\frac{9}{9}$, $1\ \frac{10}{10}|\frac{9}{9}$, $1\ \frac{10}{10}|\frac{9}{10}$, $10\ \frac{10}{9}|\frac{10}{9}$, $4\ \frac{10}{10}|\frac{10}{9}$, $6\ \frac{10}{10}|\frac{10}{10}$, $7\ \frac{10}{9}|\frac{10}{10}$, $1\ \frac{10}{9}|\frac{11}{9}$, $1\ \frac{10}{10}|\frac{11}{10}$, $1\ \frac{11}{9}|\frac{10}{9}$, $1\ \frac{11}{10}|\frac{10}{9}$, $10\ \frac{11}{10}|\frac{10}{10}$, $3\ \frac{11}{10}|\frac{11}{10}$, $1\ \frac{11}{10}|\frac{12}{10}$, $1\ \frac{12}{11}|\frac{12}{11}$. These counts were made on consecutive animals as they came from the trawl line, and must represent fairly the dental conditions of Bdellostoma in the Bay of Monterey. Two things are apparent. First, there is not the least relation between the number of gills and the number of teeth; and, second, the teeth are subject to much greater variation than the gills, notwithstanding they belong to the so-called hard parts. Size and age do not affect the dental formula.

On combining the dental formulæ of the 11-gilled and 12-gilled variations, we find the following numbers to obtain for the 86 individuals whose dental formulæ I have carefully counted on both sides of the dentiferous plate.

No. of Individuals.	Dental Formula.	Predominant Number of Teeth.
1	$\frac{9}{9}\|\frac{8}{9}$	
8	$\frac{9}{9}\|\frac{10}{9}$	9
1	$\frac{9}{10}\|\frac{10}{9}$	
1	$\frac{9}{10}\|\frac{10}{10}$	
3	$\frac{10}{9}\|\frac{9}{9}$	
1	$\frac{10}{10}\|\frac{9}{9}$	
2	$\frac{10}{10}\|\frac{9}{10}$	
14	$\frac{10}{9}\|\frac{10}{9}$	10
8	$\frac{10}{9}\|\frac{10}{10}$	
4	$\frac{10}{10}\|\frac{10}{9}$	
7	$\frac{10}{10}\|\frac{10}{10}$	
1	$\frac{10}{9}\|\frac{11}{9}$	
2	$\frac{10}{9}\|\frac{11}{9}$	
1	$\frac{10}{10}\|\frac{11}{9}$	
2	$\frac{10}{10}\|\frac{11}{10}$	
1	$\frac{11}{9}\|\frac{10}{9}$	
1	$\frac{11}{10}\|\frac{10}{9}$	
13	$\frac{11}{10}\|\frac{10}{10}$	10
1	$\frac{11}{11}\|\frac{10}{10}$	11
8	$\frac{11}{10}\|\frac{11}{10}$	
1	$\frac{11}{11}\|\frac{11}{10}$	
1	$\frac{11}{10}\|\frac{12}{10}$	
1	$\frac{12}{9}\|\frac{11}{9}$	
3	$\frac{12}{11}\|\frac{12}{11}$	

From this table we learn that more than seven-tenths of the entire number of individuals belong to three groups, and over half of these belong to the group in which 10 teeth occur in both rows oftener than any other number. We have to do with a reduction in the primitive number of teeth. So much for the California specimens.

The Chilian specimens seem to average a larger number of teeth, for Girard counted in his type specimen of 14 gills $\frac{12}{12}\|\frac{12}{12}$, while Putnam found $\frac{13}{12}\|\frac{13}{12}$ or $\frac{12}{11}\|\frac{12}{11}$ in the material he studied from Talcahuano Bay, brought to this country by the Hassler expedition, which is reported as having 10 gills. Lockington gives the formula as $\frac{10}{9}\|\frac{10}{9}$, which harmonizes with what I find to be the most abundant formula. Lacépède's example from Chili had a dental formula of $\frac{11}{7}\|\frac{11}{7}$, which is quite unusual, and indicates what we may expect from a careful study of a large series from the South American coast. I fear that the dental formulæ published in the systematic accounts of Bdellostoma are based for the most part on counts of the teeth *of one side of the tongue only*, and in this way only can I account for the bilateral symmetry which characterizes them.

The reproductive organs of Bdellostoma are extremely simple in their structure, and are composed in both male and female of a simple, double-layered plate of the peritoneum which hangs down as a fold from the dorsal wall of the body cavity on the right side of the median line (Fig. 12). The ovary occupies nearly the whole extent of the body cavity in the female. The testis occupies only the posterior part of this long fold, and in the female no eggs are developed in this region. In hermaphrodites the two divisions of this organ are

separated by a notch in the lower edge of the fold. The left organ seems never to be developed in any of the Myxinoids. In all cases, so far as my observation goes, each individual is potentially bisexual, but Bdellostoma is not much like the Myxine in changing its sex with increase of age ; certainly this condition does not seem to be so clearly expressed in Bdellostoma as in Myxine. For whereas in Mixine only the young animals seem to be males, in Bdellostoma large and presumably old animals are found to be males as well as females, and both

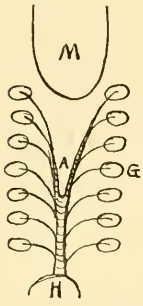


FIG. 10. — A view similar to that shown in Fig. 9, but drawn from a 7–7 gilled Bdellostoma. Lettering the same.

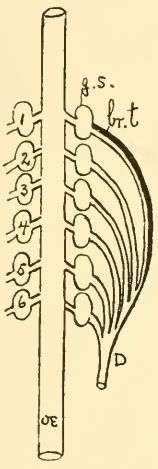


FIG. 11. — The branchial apparatus of Myxine, seen from the ventral face, to show what the changes are which have made Myxine's respiratory tract so different from Bdellostoma's. It is at once apparent that the *only structures in any way changed* are the external branchial tubes, which, by all uniting together, have only to pierce the skin at one place. *œ.*, œsophagus, *1–6* and *g. s.*, the branchial sacks. *br. t.*, the external branchial tubes of the first gill. *D*, the short duct formed by the union of all the gill tubes of one side.

sexes are found among the smallest individuals taken. The large anterior region of the peritoneal fold is always ovary, while the much smaller posterior region of the fold is the testicular region. The ovary is readily distinguished by the numerous eggs in many stages of development, while the testicular part, which is separated from the ovarian part by a notch or deep scallop cut in the border of the fold, is to be distinguished by its clear vesicles, grouped together like a bunch of grapes. Of course a crucial test of the character of the small vesicles is the presence of spermatozoa in the testicular part. Tested in this way many individuals were found to be ripe, while among the females very few were found to contain eggs in

the last stages of ripening. The explanation for this scarcity of ripe females which has been applied to the case of Myxine, *viz.*, that the pregnant females, when the egg-laying time comes on, cease to eat, and consequently are not to be taken either in baited pots or on trawl lines, must suffice for Bdellostoma until we have a better one. Other causes may be operative here, but nothing is known with certainty of the conditions of life which surround the egg laying and embryology of Bdellostoma.

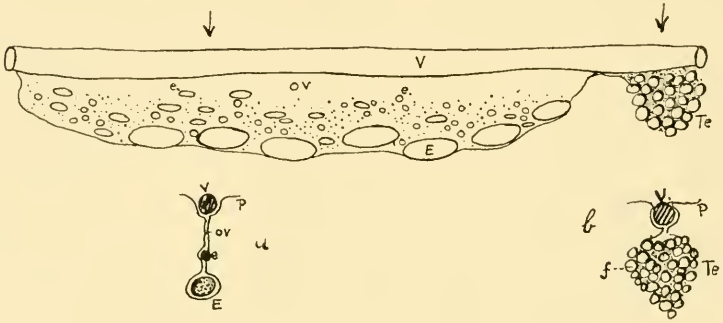


FIG. 12.— A side view of the sexual gland of an hermaphroditic Bdellostoma dombeyi. *a* and *b*, cross-sections of the gland at the points indicated by the arrows. *v*, the dorsal blood vessel, below which the gland is suspended as a double fold of the peritoneum. *p*. *ov*, ovary. *te*, testis. *E*, nearly ripe eggs. *e*, very young ova. *f*, testicular follicles.

Some individuals of Bdellostoma are truly hermaphroditic, having at the same time a genuine testis with ripe sperm and an ovary with eggs nearly or quite ripe. These individuals are rare, so far as my experience goes, but others with the two organs in a more unequal state of development are more numerous. However, by far, the largest number of individuals are genuinely male or female. Males are much more numerous than the females, but this statement applies only to the catches which I had the opportunity of examining. It may be merely a coincidence, or on the other hand it may faithfully represent the true conditions of things in the Bay of Monterey. All things considered, I believe a preponderance of males represents the ordinary condition.

The sex of 209 out of 354 individuals was noted, and they

are distributed between the males, females and hermaphrodites in the manner indicated by the following table :

Of 101 individuals having 11 gills on both sides,	.	61	were males,	37	were females,	and	3	hermaphrodites.
Of 26 individuals having 11 gills on one, 12 on the other side,	.	14	"	12	"	"	0	"
Of 163 individuals having 12 gills on both sides,	.	94	"	66	"	"	3	"
Of 11 individuals having 12 gills on one side and 13 on the other,	.	8	"	3	"	"	0	"
Of 8 individuals having 13 gills on both sides,	.	5	"	3	"	"	0	"
Totals,		182		121			6	

The sexuality of the Bdellostoma does not appear to depend upon size or age, for of 10 small individuals (under 15 inches) there were 7 males with gills, $\frac{12}{12}$, 2 females with gills, $\frac{12}{12}$, and 1 female with gills, $\frac{11}{11}$.

Of 15 large individuals (over 20 inches) there were 8 males with gills, $\frac{12}{12}$, 1 female with gills, $\frac{12}{12}$, 5 males with gills, $\frac{11}{11}$, and 1 male with gills, $\frac{13}{13}$.

In 1875, Anton Dohrn, the founder and talented director of the Zoölogical Station of Naples, first clearly defined the *hypothesis of degeneration*, and fully illustrated its application to the *vertebrata* in his famous paper, "Origin of Vertebrates." Dohrn corrected the then prevalent ideas, according to which all the simpler forms of animals, anatomically considered, were to be looked upon as more primitive and as representing ancestral stages in the development of the groups to which they belonged. He was not fortunate in his selections of cases of degeneracy among vertebrates, as I hope to make clear to you. As a typical case of true degeneracy we have the well-known case of the Tunicate, which during its development passes through a stage which, according to the "*biogenetisches Grundgesetz*" must be considered to be strictly vertebrate in its morphological characters. These vertebrate characters are not retained by the Tunicate, however, but are absolutely destroyed during the growth of the larval body, so that in the adult condition there is nothing left to ever *remind*

one of the vertebrate type of organization, and the body ever after remains in this inferior condition of structure, though it produces germs which in their development attain the higher level of vertebrate organization, only to sink back to the lower level of Tunicate structure.

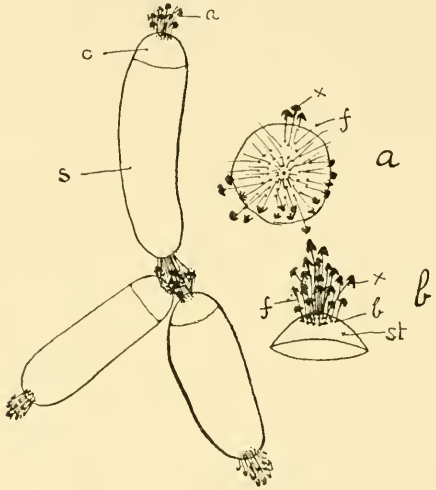


FIG. 13.—A view of three ripe eggs of Bdellostoma dombeyi, showing their natural size, the variations in shape, the manner in which they hold together by means of their anchor hooks, and the position of the suture which allows of the ready separation of the cap from the body of the egg membrane. *a*, an end view of an egg cap. The anchor filaments have all been radially spread to show the spiral arrangement of the filaments upon the cap surface. *x*, the anchor head. *f*, the filament. *b*, a side view of another cap. *st*, the cap membrane. *b*, the row of enlargements forming the bases of the filaments, *f*. *x*, the anchor heads. The central dot in *a* shows the position of the micropylar funnel which leads into the micropylar canal. The egg membrane is composed of a thin genuine vitelline membrane and a thick corneous follicular (granulosa) membrane, and the anchor threads are hollow evaginations from the end parts of the latter. Natural size, *a* and *b* × 2 diameters.[1]

[1] After a study of the development and the adult condition of the egg membranes in Bdellostoma dombeyi I am certain that Cunningham is in error in calling the egg membrane of the Myxinoids a vitelline membrane. The corneous part of the egg shell is cellular in nature and the cell remnants are to be seen in the ripe egg shell. The anchor hooks are formed by the evaginations of this granulosa layer in late stages of egg life, due to the outgrowth of solid rods of the vitelline membrane. These rods appear to be resorbed before the ripening of the egg and thus the anchor filaments are left hollow. The Myxinoid egg is thus very much like other fish eggs as regards its envelopes. An illustrated account of these facts, among others is ready for the press.

Thus it is that the evidence in favor of progressive simplification of structure (which is all that is meant by the term degeneracy) depend upon *two* factors:

(*a*) The comparison of the anatomy of the fully developed degenerate animal with that of its supposed congeners. In this way we can often be sure that the less complex animal does not represent any of the ancestral stages common to its nearest relatives. This is the anatomical method, and (*b*) by the study of the embryological development of the forms under consideration. This is the embryological method, and it is very generally admitted to be conclusive whenever it can be shown that either the embryonic or larval stages of the animal under consideration possess a higher degree of morphological differentiation than is possessed by the adult form. *e. g.* barnacles (Cirrhipedes), Tunicates. In many instances the embryological history may be, and is, so shortened as to give no trace of a higher stage of existence having ever been enjoyed. *e.g.* many Tunicates. Even from this very short review of the degeneration hypothesis, it is evident that we must conclude that *à priori* there is much reason to suppose that any given animal form may have developed from a more highly organized ancestor by a process of simplification as to assume that it has developed from a relatively simpler form by an unbroken process of complication, and that our only means outside of morphological research is to be derived solely from the life habits of the animal, *i.e.* whether they are such as to favor degeneracy or not. In saying that there is much reason, on *à priori* grounds, to assume that an animal has degenerated or developed downward to its present condition as that it has developed upward, I certainly cannot acquiese in the view expressed by Dohrn and adopted by Lankester that there is as much probability in favor of the one process as of the other, for it is evident that cases of degeneracy must be much less common than cases of progression, otherwise the Law of Agassiz, fully established by Darwin, would not be so easy of observation in embryology and palæontology. The law of progressive differentiation is so universal in its application that we are on safe ground in always considering any existing form

as the result of a series of progressive changes, or viewing any fossil form as the highest of its kind up to the time of its existence, unless in either case there is positive proof to the contrary; for the biogenetic law lays the burden of proof upon the degenerationist. Life habits, favoring simplification or obliteration of structure, are parasitism, sessile or fixed life, burrowing and hiding in crevices and holes, and indiscriminate and diffuse food habits, while free and unrestricted motion within the aqueous and ærial oceans upon and over their bottoms, involving the pursuit of fleeing prey and the struggle with other forms for its possession and for life itself, are highly favorable to increasing complication of structure. It is at once evident that we cannot take existing vertebrates and group them either in an arbor-like or ladder-like geneological arrangement, and at the same time express the true relationships of the many modifications of structure which have been produced. This brings us to the consideration of the question of the classification of Bdellostoma, and to this I shall return after throwing what light I can upon the conditions under which Bdellostoma exists and the probable relations which this fish bears to these conditions of its environments.

What evidence can we bring forward to show that "the small and degenerate group *Cyclostomata*" as Lankester[1] calls them, *are not degenerate Vertebrates?* In previous publications I have pointed out some of the facts, and I am sorry to say they have not been accorded that consideration which they merit as anatomical facts. In 1889[2] I showed that "*the higher sense organs of the Cyclostomata are all paired, since the nose (i.e., the nasal or olfactive epithelium) exists in the embryo as well as the adult in the form of two circumscribed areas lying on either side of the median line, each of which receives the entire nerve supply afforded by the olfactory nerve of its side.* I then ventured to predict that the Myxinoids would show the bilaterally symmetrical or paired nasal areas as distinctly as Petromyzon does. I can now state that my conclusion was well founded, for in Bdellostoma we have the nose divided into right and left halves

[1] Reprint Article, Vertebrates, *Ency. Brit.* ed. 1887. London, 1890.

[2] Concerning Vertebrate Cephalogenesis, *Journal Morphology*, IV, 1890.

by a median plate which is the homologue of the median raphe of Petromyzon. In Figs. 7, 8 is shown this important feature as seen from the ventral surface (inner face) of the nasal organ. As concerns Bdellostoma's eye, we know very little of its adult structure and nothing of its development, and the conclusion that it is a degenerate organ in lacking a lens, choroid, etc., and being entirely below the surface of the body and under the skin, is purely hypothetical, for it can just as well represent a stage in the phylogenetic development of vertebrates. I believe such to be the truth. The undegenerate nature of the ear I have already established in another publication.[1] On the basis of our knowledge of the higher sense organs we may well ask: Why are the Myxinoids *primitive fishes?* instead of "Why are they degenerate vertebrates?" The following considerations enable us to understand why they are primitive and not degenerate:

1. Their unusually great geographical distribution indicates that they are descendants of a very ancient stock.

2. They are in a morphologically undifferentiated condition, as compared with all other groups of craniate vertebrates and their several organs show no certain traces of degradation of structure. In this regard I have examined the following organs: skin (here the absence of surface sense organs, scales, except the teeth, and a lateral line is noteworthy and entirely unexplained; they are not degraded but *have entirely vanished from adult life*), muscular system, skeleton, vascular system, alimentary system, urino-genital system and the higher sense organs, eye, ear and nose. Of these latter the two last are normal stages in the development of the vertebrate stock, and so far as I have examined the structure of the eyeball, its lack of lens and eye muscles and its small optic nerve, I have found no conditions which are not harmonious with the view that this eye has simply never been highly developed.

The degeneration hypothesis fails completely to account for Myxinoid anatomy, and the habits of the animal give no grounds for this assumption. Certainly the natural conditions surrounding the life of the animal as far as we know them, are

[1] Contributions to the Morphology of the Ear, *Journ. Morphol.* VII, 1892.

not such as to cause the extensive retrogressive development which they are supposed to have undergone and which it is assumed has affected nearly all of the organs of the body.

It seems to have become a settled belief among the large majority of zoölogists of both morphological and systematic proclivities that the number of gills found among vertebrates never rises above eight pairs in existing forms. The few who have recognized the true state of the case have had little to do with the education of the younger men, and so the error has continued to be taught. Lankester[1] wrote in 1890 — regarding the gills of vertebrates, "The pharyngeal slits follow closely upon the mouth, and in existing *Craniata* never number more than eight pairs." Wiedersheim[2] gives seven pairs of gills as the largest number occurring among craniate vertebrates, while Claus, Huxley, Jackson-Rolleston, Hertwig and others give the number varying from 5 to 8, but never greater than 8. They evidently confine themselves to Müller's statement concerning the number of gills (6–7) in Myxinoids and to the known facts concerning Petromyzoa (7) and the Notidanidae (6–7). In 1854 Charles Girard described a fish from the coast of Chili which he called Bdellostoma polytrema, and which he found to have 14 pairs of gills. In 1873 Putnam found that the Bdellostomas of the Hassler expedition, collected off the coast of Chili, had 10 pairs of gills. Later on Lockington (1878) described a similar fish from San Francisco which had 11 pairs of gills. These are the only original observations, with which I am acquainted, which give the number of Myxinoid gills greater than the accepted numbers (6–7). Yet these facts have been many times confirmed by such men as Gunther, 1873, Gill, 1880, and Jordan and Gilbert, 1882, who gave the number of gills of the Pacific Bdellostomid as 11–14. Notwithstanding all these published accounts, morphologists have completely overlooked these facts, and while speculating on the ancestral number of gills of vertebrates, have depended for the most part upon the embryological stages of Elasmobranch and Teleost fishes.

[1] *Ency. Brit.* Article on vertebrates.

[2] Grundriss d. vergl. Anat. Jena, 1893.

I have already referred to this matter in connection with the brief description of Bdellostoma's gills, but it is sufficiently important to call for a more detailed account, in which I shall try to bring the branchial apparatus of Bdellostoma into harmony with the primitive condition of the same organs in Branschiostoma.

Having shown that there is a great degree of variability in some of the organs of Bdellostoma, it will be in place to seek for the conditions which keep up this variability, — for the conditions must be *present* conditions and in no sense past ones. Three conditions suggest themselves as probably concerned in keeping up this variability among the Bdellostomas as a group of animals of common descent and as *families of individuals* living under common conditions in circumscribed areas. These are Geographical Distribution, Panmixia and Hermaproditism.

The geographical distribution of Bdellostoma cannot be satisfactorily accounted for by assuming several separate creations of these animals, for though so widely distributed on the floor of the Indo-Pacific oceanic depression, all its morphological characters and its life habits point to its origin from a single ancestor at some place in the South Pacific ocean. Admitting the common descent of the varieties of Bdellostoma, the phenomena connected with the geographical distribution of animals in general lead us to conclude that Bdellostoma has existed for a very long period of time and that it has been but little modified during this long period. We do not know that it has always been as variable as it is at present; but if it has been, it is a most remarkable case, since *ordinary animals would have become well differentiated into distinct species during the process of dispersion over such a great territory* and the consequent operating of more or less changed conditions in different ways upon different parts of the variable organism during the great period of time necessary to accomplish this dispersion. Bdellostoma has been widely distributed, and while the conditions in different parts of the territory which it inhabits can hardly be held to be identical, still the sea bottom is far more stable in its temperature and other features produc-

tive of variation than the shallower waters of inland basins or the surface of the land itself. Hence *so far as the effects of geographical distribution are concerned, they may be said to have had very little or almost no effect upon the anatomical structure of the animal.* This conclusion is fully sustained by the fact that in each locality Bdellostoma shows similar variability in the same organs, while individuals from widely separated localities show variations which are frequently numerically identical. The variability is of the same kind and comparable in degree in individuals from all the localities yet studied. This is by no means a satisfactory presentation of the effect of geographical distribution, but it must suffice for the present, and until our knowledge of the distribution of the animal has been more fully elucidated.

What effect should these facts have upon our ideas of the classification of Bdellostoma? Most authorities are inclined to look upon the difference in the number of gills as a sufficient ground for the establishment of distinct genera. That this character has not a generic value it is needless to state, for reasons already sufficiently repeated. But it would be convenient to recognize these several varieties by some name, if possible, and, according to the present system of nomenclature, there are two opportunities, — one for a specific, the other for a variety name. Is the gill variation a sufficient specific character? I think not, for the reason that we have all *grades of the variations*, and the further reason that they all belong to one colony of freely intercrossing individuals. It appears to me by far the best plan to recognize one genus of Bdellostomids, with one species composed of the several varieties, and I propose that we return to the first satisfactory name that was applied to our animal. The first account of this animal which we find in the literature is that of Lacépède, who described it from a dried skin sent from the Chilian coast. He named it *le Gastrobranche dombey*.[1] Later, in 1815, Home described the gills of a Heptatrema which he obtained from

[1] Gastrobranchus is the old name applied to Myxine, and these forms were kept with Myxine till Duméril put them in the genus Heptatrema, which of course gives this name priority over Müller's Bdellostoma.

Banks' South Sea collection. Johannes Müller published his well-known monograph on these forms in 1834, and applied the name Bdellostoma to the forms from the South Sea, Cape of Good Hope, and Chilian coast, making species on the basis of the number of gills. He called the Chilian form B. dombeyi, and made five species in all. In 1854, C. Girard described a fish from the Chilian coast with fourteen gills, and called it Bdellostoma polytrema.

In 1878, Lockington described the form found in San Francisco Bay as B. stouti, and Gill changed the name in 1880 to Polistotrema stouti, which has again been changed to Polistotrema dombeyi. Retaining the name polytrema for the Chilian form, he changed it over to his genus Polistotrema, which he says is characterized by 11 to 14 gills. These accounts all refer to the varieties of what I shall call Bdellostoma dombeyi, adopting Müller's genus, on account of the inapplicability of Lacépède's Gastrobranchus, and of the inappropriateness of Cuvier's Heptatremes, which could only be used for the seven-gilled form or variety. Bdellostoma, the generic name proposed by Müller, is satisfactory in every way, and we may well use it.

The specific name dombeyi, applied by Lacépède in 1798, is satisfactory in that it avoids all difficulties of a morphological kind, and perpetuates the name of the discoverer of this remarkable animal. Bdellostoma dombeyi is the name under which are gathered all the variations we know of in the branchial and dental systems and the correlated organs. For the purpose of distinguishing these varieties, we may use either Latin variety names or a simpler numerical termination. If we choose the Latin names, we meet with difficulty in supplying all of the *heterotremes* with sufficiently exact names, for some are 6–7, others 11–12, and still others 12–13-gilled, and no one knows when or where more of these *heterotremes* may be found.

Gastrobranchus dombeyi (Lacépède), 1798.
Heptatremes dombeyi (Cuvier), 1829.
Bdellostoma " (Müller), 1834.
" " (Gray), 1851.

Bdellostoma polytrema (Girard), 1854.
" " (Günther), 1870.
" " (Putnam), 1874.
" Stoutii (Lockington), 1878.
Polistotrema dombeyi (Gill), 1881.
" " (Jordan, Gilbert), 1882.
Bdellostoma forsteri.
" cirrhatus.
" hexatrema.
" heterotrema.
" heptatrema.

I shall, for my own convenience, hereafter use the numerical method of designating the varieties; and, unless sufficient reasons be brought against this style of name, I would urge its adoption on the ground of convenience, to go no further.

Bdellostoma dombeyi, 6 gills.
" " 6–7. } Indicating the sides of the body upon
" " 7–6. } which the respective numbers occur.
" " 7.
" " 10.
" " 11.
" " 11–12.
" " 12–11.
" " 12.
" " 12–13.
" " 13–12.
" " 13.
" " 14.

Physiological.

From previous experience it seemed to me very desirable that physiological experiments should be tried upon the ear of some vertebrate with the simplest existing type, and, if possible, upon an animal which lacked fins, — *i. e.*, paired appendages, which in all fishes are specially used in maintaining the equilibrium of the body. I felt that by securing these conditions, we should be able to get much cleaner responses or, in any case, safer results from operations on the ear, for the presence of the paired fins — especially the pectoral fins — complicates the reaction to ear operations by introducing into observable phenomena mechanical factors whose influences have not been carefully enough studied and

allowed for. The desired vertebrate was found in Bdellostoma, which thus presented me with material for both morphological and physiological investigations. Physiological operations on the ear of Cyclostomes have been tried on Petromyzon only, so far as I know, and then they were abandoned without obtaining results, on account of the difficulty of the operations. The first operations I performed were on anæsthetized animals; but I soon abandoned the use of anæsthetics for several very sufficient reasons.

My method of operation was the following: In order to hold the extremely slimy and slippery animal, I used large sheets of blotting-paper, such as botanists require for drying plants. Bdellostoma was taken from the aquarium, and at once rolled up in the sheet in such a fashion as to hold it extended during the operation. One sheet makes a roll sufficiently stiff to retain the fish perfectly. A stout needle was thrust through the skin at the side of the mouth, and another through the skin and muscles of the tail, and both forced into the table at such a distance apart as to prevent the fish from squirming or crawling out of the paper cylinder. The blotting-paper was removed over the region of the ear, and an incision made through skin and muscle, exposing the cartilaginous ear capsule. The top of this was shaved off with a sharp scalpel of suitable shape and size, and the auditory nerves cut inside the ear capsule, either with a scalpel or scissors. The columella was then cut, and the ear lifted out with forceps or with a small bent needle. An examination was made with a lens to see if any part of the ear or auditory nerve had been left in the capsule, and any such fragments were removed before the cut in the skin was sewed up. This done the animal was unrolled into the aquarium, and its motions watched. The whole operation does not require more than two minutes, and the fish does not appear to suffer in the least from suspended respiration, so far as I could make out.

A fish thus operated upon, with both ears removed, will swim from the moment it is placed in the aquarium like a normal fish. In some cases the creature will tie itself into a knot, with the evident purpose of removing the irritation

from the skin of the head, but it soon leaves off such attempts, and settles down on the bottom of the aquarium, coiling up in the normal fashion, or else it will continue to swim about the tank for a time. In case only one ear is taken out, the animal may (it does not always do so) swim with the injured side lower than the other, or may even roll as it swims — *especially if it is excited to swim rapidly;* but like others, it will settle to the bottom, and rest normally in its coil, coiling either away from or toward the injured side. I thought I could notice in some cases a tendency to coil away from the injured side; *i.e.*, the normal side did the work of coiling; but the case is by no means clear that this coil is done oftener than the other.

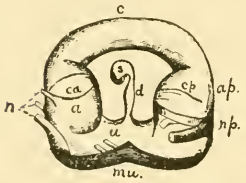


FIG. 14. — The right internal ear of the Hagfish (*Myxine glutinosa*), seen from the inside or cerebral face. Figure after G. Retzius. The figure represents the ear somewhat enlarged, and does not show the shape or exact positions of the contained sense-organs.

a	Anterior ampulla.	*d*	Ductus endolymphaticus.
ap	Posterior ampulla.	*mu*	Macula utriculi et sacculi.
c	Anterior and posterior canals.	*n*	Nerve branchlets.
ca, *cp*	Ampullar ends of the same.	*u*	Utriculo-sacculus.
		s	Sacculus endolymphaticus.

Now, on the hypothesis that the ear is the specific organ of the equilibrative sense, it might be argued that the removal of one ear does not necessarily so powerfully affect the equilibrium of the body as to destroy even the control of the injured side; for the uninjured side is able to take upon itself the functions of the injured organ in part at least. If that were the case, when both ears were removed we should certainly expect to see the animal lose control of its body; but, as I have already said, the animal is *even better off with both ears removed than with one ear removed, for all traces of equilibrative disturbance disappear at once on the removal of the second ear.*

How can the semi-circular canal with its two ampullae be the special organ of dynamical equilibrium and the utriculo-sacculus with its sense organs the special organ of statical

equilibrium when the animal maintains its perfect equipoise without them? If in higher forms each canal appreciates movements in its own plane and by definite functional combinations of two or more canals is able to mediate all possible rotational movements, what function has the single canal in Myxime, or the two canals in Petromyzon? Both of these creatures are subject to the same mechanical conditions in their progress through the water that the higher vertebrates are. The fact that they do not have the canals proves that vertebrates can swim as admirably without this apparatus as with it, and such being the case, what was the *physiological* incentive which lead to the production of the other canals? I have elsewhere given a sufficient mechanical explanation of the genesis of the canals, but I have yet to learn of a sufficient physiological one. Who will add to our knowledge in this particular?

It is said that the semi-circular canals in man, for example, are delicate organs whose special function is to take cognizance of all the motions of the body in their respective planes, and that they thus control either singly or by various combinations with the other canals of the opposite side of the body, all possible movements of the body.

One may readily disprove this assumption by a very simple experiment. Have some person with normal ears and normally developed muscles swim for a distance on his back with closed eyes — first with his hands and arms alone, keeping his legs straight, and second with his feet alone, folding his arms on his breast. The average man is stronger on the right side of his body than on the left, and consequently when he pulls himself through the water with his arms alone — eyes closed — he unconsciously pulls himself to the right and swims in a circle — (*Reitbahn bewegungen of German physiologists!*) When he swims with his feet alone he pushes himself through the water and consequently pushes himself over to the left side and swims in a circle in an opposite direction to what he did before (*circus movement to the left!*) and he is, so long as his eyes remain closed, unconscious of the fact that he is moving to one side *i.e.*, diverging from a straight line — but he becomes

conscious of this fact the moment his eyes rest upon something which enables him to orient himself, and yet this normal individual has six organs in his head which are there for the special purpose of controlling of his movements in space, so say the physiologists. His two eyes are of more service to him than are his six canals.[1]

It may be objected that the canals are not intended to take cognizance of such minute changes in position in space as are involved in swimming around in a circle; but if they cannot perceive such changes of position when swimming, how can they perceive them when on a rotating table (*e.g.*, Crum. Brown's experiment). It would be a great service to determine the minimum amount of angular motion of which the canals can take cognizance, in order that we may know how much to expect of our ear canals in this way.

It may be objected that the man in the water on his back is in an unusual position and consequently his canals do not work with sufficient delicacy on account of the unaccustomed pressure, etc. But it is a necessary consequence of the assumption that the canals are special organs of equilibrium, that they should work in any position; otherwise what were the use of them? Other organs of specific function work in all positions of the body. The eyes see, the nose smells, the ears hear, the brain thinks, the heart beats, and so on *ad infinitum*, all except the ear canals, whose special work is to cognize just such unusual spatial conditions, and according to the hypothesis, the only organs in the body having this function, — they fail of their function when most needed. In the experiment with the rotating table the canals are subject to even a severer test of very small angular motion and are supposed to be able to operate very precisely, in some cases.

[1] Experimental rides on the great Ferris wheel at Chicago during the Columbian Exposition proved that a perception of the direction of the motion of one's body in space was impossible unless one made use of the eyes. I found that the upward motion could not be detected by the ears alone. Without the evidence of one's eyes one seemed to be standing still upon a trembling platform, and the while was really making a great circle through the air in a geotropically oriented position. It is notorious that balloonists have difficulty in determining whether they are moving upward or in any other direction when sight is hindered, and that it is impossible to detect motion in the midst of a cloud.

I think this swimming experiment gives very satisfactory evidence to the effect that the semi-circular canals in man do not function as organs of dynamical equilibration. At the opposite end of the scale — Bdellostoma is certainly not dependent upon its ear for the equilibrium of its body. I may say in conclusion that I think Professor Ewald has made a great discovery in showing how intimately the auditory nerve is related to the musculature of the body and in proving that some of the complicated phenomena which follow section of this nerve and of its branches are fully accounted for by the fact of the lessened muscular tonicity of the injured side and not on the ground that these phenomena indicate the special functions of the several parts of the ear.

EIGHTH LECTURE.

THE INFLUENCE OF EXTERNAL CONDITIONS ON PLANT LIFE.

W. P. WILSON.

If you open your eyes and look carefully about, as you are traveling from place to place, you will easily see that there are very many differences between the plants of one region and those of another. In the high mountains you will find many thick-leaved plants, such as the Rhododendrons and Sedums, and quite a number of peculiar forms not seen lower down. Many of these may have a stunted, gnarly or dwarfed look, quite strange to the vegetation at the sea level. In still another region the plants may have lost much of their natural grace and beauty of form. They may look rigid and stiff, with small thickened leaves or none, with short thickened stems, or round and consolidated forms such as we find in the Cacti or Euphorbias. These are the tenants of desert regions.

Again, if you happen to go south into the tropics, sheltered and shaded by immense thin-leaved trees, with the waving green of the palm and the vine, you will find dense masses and banks of dark green foliage belonging to Ferns and Selaginellas, with climbing plants of various kinds intermingled.

About your own door may be quite as interesting forms as in more remote districts, only you are accustomed to them and do not see their peculiarities. You may look out on pines and oaks, on a great diversity of small plants, with the Indian pipe, a partial parasite, and a few ferns which can bear more wind and cold than most of their relatives, plenty of mosses and lichens, and, in fact, representatives of all the great plant families.

The ancients looked upon the whole animate world as having been directly created by some higher power, and believed that each species indicated a distinct and individual creation. They also observed these differences between the plants of different regions, and, in fact, between those of the same region. They thought that all living organisms were created to fit the special location and conditions under which they existed. They believed that water animals and water plants, for example, had always been and would always remain such; that the desert region had always been a desert region, and that the Cactus had been created to fit it. We are not obliged to go very far back in the history of our own times to find eminent naturalists who have strongly advocated this view. No less a man than Louis Agassiz was one of the most strenuous defenders of this theory. He believed that species of both plants and animals were invariable, living and dying in form and color and habit just as they were created. And there were many other naturalists the world over who held to this doctrine of special creation. It must not be forgotten, however, that though their views attained no prominence, such men as Lamarck had at different times indicated a strong belief in the variability of forms.

During the last twenty years there has been a great revision of thought in regard to these subjects. At present all naturalists believe not only in the possibility of a continual change in both plants and animals, to fit them for varying conditions, but also in the gradual growth and development of very diverse changes in climates, rendering equal modifications in both fauna and flora absolutely essential to their continued existence. Since great changes have always been slowly taking place in the earth's surface and climate, and since present vegetation has always been subject to these gradually varying conditions, it must have adapted itself, with a slowness commensurate with the earth's changes, to the ever-appearing new conditions. In this way, we think, can be solved the problem of the infinite variety of vegetation.

By experiment we may determine the two factors underlying plant variation. On the one hand the laws of inheritance are

ever holding the plant invariable, while the external forces are ever pushing it toward variation. In this lecture we will disregard entirely the interesting phenomena which relate to that internal force the manifestations of which we call inheritance, and try to discover how much the plant world is moulded and shaped by what is exterior to it.

If you are inclined to think about all these differences in the vegetation of the many regions through which you have travelled you will soon see that where there is great change in the appearance of the plants, in passing from one place to another, there is also an equally marked difference in the surroundings or soil or climate. You will soon find yourself always looking for the one when you have found an expression of the other, *i.e.*, if the plants give you a new flora, you seek at once to find the cause in external conditions; or, if you have great external difference in surroundings and climate, you expect this to be at once reflected in the varying vegetation about you.

Let us study a few plants a little more closely, and see if we are able to determine on what this variation depends and how rapidly it may take place. I shall assume at the outset two sets of causes, both of which may be active in bringing about variations in plants, the one wholly external, such as light, heat, moisture and the like, and the other internal, and concerned with the laws of inheritance, about which we know so very little.

Let us for our present purpose consider only the external influencing conditions, for I believe that they are much more potent and determinative than the laws of inheritance. They may be enumerated as follows :

1. *The Water Supply.*— This is more important than any of the following points, as it is intimately connected with the very vital process of organizing materials for growth and their transport throughout the plant.

   If the water supply is cramped and too little, we have immediately before our minds the desert plants.

   If the water supply is too great, equally important changes take place.

2. *The Light Supply*— Here again we may have too much or too little, either condition leaving its strong impress on the plant.

3 *Altitude* is a third factor in the growth of plants, which strongly influences the light, the moisture and the temperature, as we shall see in a later discussion.

4. *Temperature.*— Temperature and light, together, govern largely the transpiration of water from the plant.

The character and position of the leaves are often wholly adapted to both the conservation and the loss of water by transpiration.

5. *The Food Supply* produces the most fundamental changes in the life of the plant, strongly influencing not only the production and character of the flower and the fruit, but in all probability determining the sex itself.

6. *The Influence of the Sea upon Plant Forms.*— It will be seen that quite as remarkable changes come to the sea as to the desert plants, and in truth based upon similar reasons.

(1) Let us first consider the water supply. You will readily bring before the mind's eye the picture of desert plants, which represent in their modifications a lack of water. But you will not so easily call to remembrance plants which have undergone changes from an oversupply of water. Yet there are many of them even in your own neighborhood.

The Bald Cypress (*Taxodium distichum*) grew in the present Arctic region before the Glacial Epoch, in company with oaks, maples, willows, the Redwoods (*Sequoia gigantea*, *S. sempervirens*), the Gingko tree (*Salisburia adiantifolia*), *Torreya*, and *Glyptostrobus*. During later changes in climate these trees were driven from their home and travelled down widely different lines into the South. For reasons which we are unable to understand, the *Sequoia*, or Redwood, descended along the California coast, the *Glyptostrobus* and the *Salisburia* or Gingko tree, down the coasts of China and Japan, the *Torreya* and *Taxodium*, or Cypress, to the eastern and southern parts of North America. At present the Bald Cypress, to which I wish especially to call attention, grows only along the water-

courses and in the swamps of the south-eastern part of the United States. It seeds itself naturally nowhere outside of areas which are for several months during the year under water. This tree belongs to the Pine family, but has some very marked peculiarities which make it differ strongly from any of its relatives.

The tree shown in Plate No. 1 stands normally surrounded by water. Many root-like projections are seen underneath and around the tree, which are popularly known as cypress knees. These are all connected with the root system below the water. They have extended their growth upwards until they are sure to remain in the air at the ordinary level of the water during most of the year. They are in such numbers that wagon-loads of them could be taken away from one tree. From many careful experiments made on the growing seedlings, it has been determined without doubt that these knees are organs of respiration. All dry-land trees secure free oxygen from the soil to carry on the oxidations needed in root growth. This tree growing in water, which holds much less oxygen than the soil, is unable to draw its full supply from the medium surrounding the roots. To fill this want, these knees are pushed up out of the water into the air as aerating organs. Although the plant does not now grow normally on dry land, yet when planted there it thrives as it once did in previous geologic ages in the Arctic regions. Plant two sets of seedlings, the one in dry soil, the other in soil flooded with water ; the first will show no signs of the root-aerating organs, while the second will develop them in abundance, as small vertical roots pushed above the surface of the water.

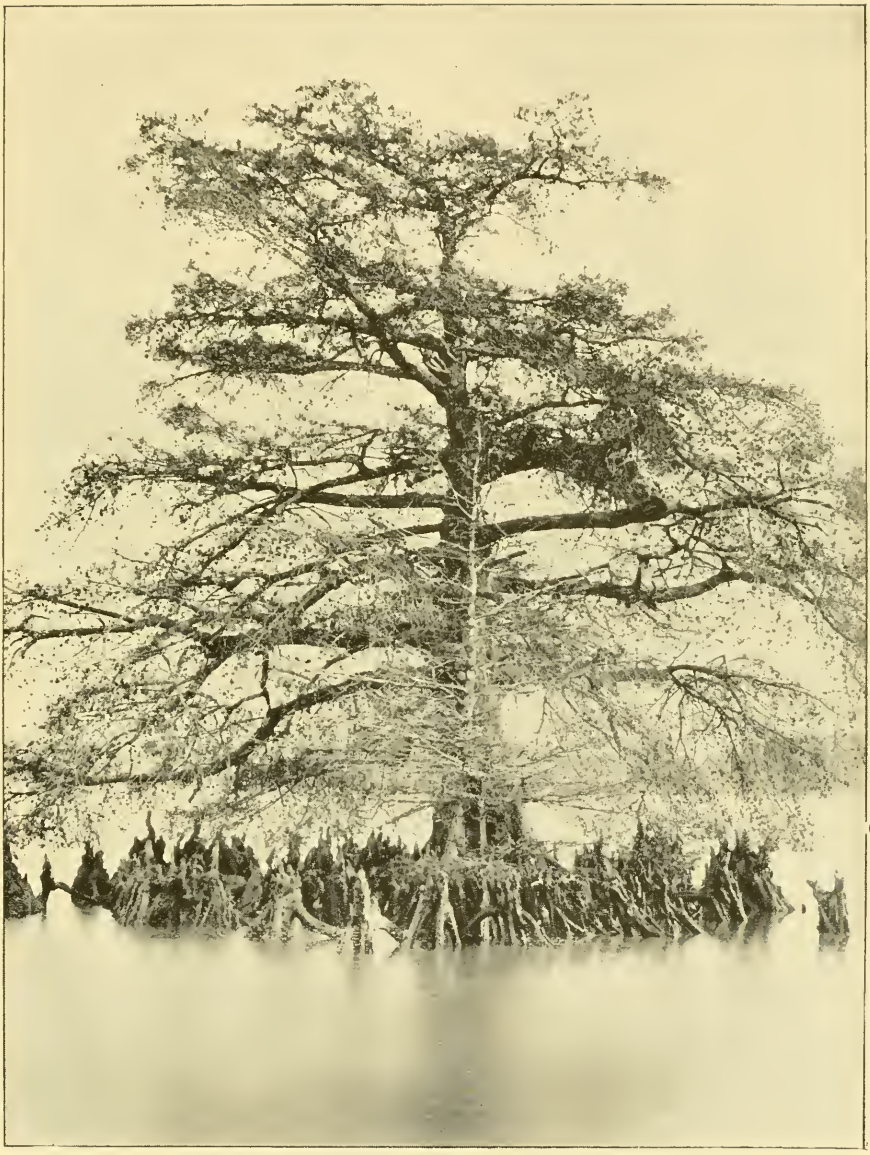
In the water growth, the branches of the Cypress are spreading and the top flattened, a marked departure from the most striking characteristic of the Pine family, to which it belongs. When grown, however, in dry soil, in our parks and public grounds, it reverts to the normal type. The branches are short and make a sharp angle with the main stem, and its cone-like form is as pointed as in any of the tall conifers. This will be seen in Plate No. 2, photographed from a tree planted in Fairmount Park, Philadelphia.

It will be observed, too, that there are no signs of knees underneath.

Plate No. 1 represents a water form from the James River. The base, in this form, is always very much enlarged. This enlargement is a part of the aerating system needed to secure the necessary oxygen, and is, of course, entirely absent from the land form.

The departure from the sharp, cone-like form natural to the conifers, is due to the difficulty in obtaining both air and food from the water. This lack of nourishment shows itself in the dwindling, depauperate and dying branches of the upper part, since that part is most remote from the food supply.

In some of the lakes of Southern Florida it now and then happens that a cypress tree has obtained a foothold in much deeper water than is its normal habit. Here all of these water peculiarities are greatly exaggerated. The trunk of the tree produces an immense cone, the top of which points up to the surface of the water and ends with a few flat sprayey branches. The base of the cone may be forty or fifty times the diameter of the top, from which come the few straggling branches which project above the surface of the water. There are at its base innumerable knees. In a tree having the height of twenty-five or thirty feet, the cone below the water will represent a little over one-half, and the branches above the other half of its altitude. Plate No. 3 represents such a tree in South Florida after the waters of the lake had been drained away. The knees, being of no further use, quickly rotted and disappeared and are not shown in the photograph. Originally the water was high enough to touch the lower branches, and the tree eked out a miserable existence, struggling hard in the deep water for both air and food. The difficulties under which it labored developed it into a great monstrosity. It is an extreme expression of excessive water supply. Compare its short, cone-like trunk, entirely immersed in water, with what may be called the normal development now found only in our parks (Plate No. 2). In this form the trunk is tall and slender, with branches towering to a very sharp point. The water form has acquired its knees and greatly enlarged



NO. 1. — TAXODIUM DISTICHUM RICH. — JAMES RIVER, VA.

NO. 2.—TAXODIUM DISTICHUM RICH.—FAIRMOUNT PARK, PHILA.

No. 3. — Taxodium distichum Rich. — Kissimmee City, Fla.

base for purposes of respiration. Its flattened cone is the result of the bad nutrition. The land form, dropping into its native dry earth habitat, does not develop the knees and enlargement, because its oxygen supply is ample. Its nourishment, too, is sufficient, so that it will have as a result of these normal, favorable conditions, the tall, sharp, cone-like form natural to the pine family. Doubtless the cypress has been millions of years adapting itself from its dry land conditions to its watery surroundings, and the most interesting fact in this connection is its readiness to fall back into its old habit of growth, and even in the first generation on land to lose every trace of these wonderful acquisitions.

There are many other plants which have acquired equally remarkable organs in the same way. Some of the mangroves of the Florida coast such as *Avicennia nitida* and *Laguncularia racemosa*, growing in the ooze between tide-waters, have developed vertical roots which project out of the mud by hundreds under each tree and are exposed to the air at every low tide. These organs aerate the plant as the knees aerated the cypress. They are much more highly organized, being covered on the parts above the mud with great numbers of large, open lenticels.

It is not necessary for us to go so far from home to find numbers of plants which have adapted themselves, through special aerating organs, to a change from dry land to water growth. One of the most common is *Decodon verticillatus*, which develops over the surface of all its roots an extremely thickened, corky, air-holding layer. A similar development covers the branches when they happen to drop into the water.

Let us now see how plants are modified when they lack water. If the supply is inadequate, the plant makes an attempt to conserve the little that it has. As the surface of ordinary plants allows large quantities of water to escape by transpiration, and as this loss is largely proportioned to the amount of surface exposed, such plants usually lessen this surface by making the leaves smaller and thicker, or by losing them entirely, by shortening the branches and by consolidation generally. As the light and the heat of the sun increase

transpiration, the surface of the plant may become for protective purposes hairy; the cuticular and epidermal layers may be thickened; the interior air passages in the leaf which communicate with the surface may become obliterated through consolidation of the cellular structure; and, in certain cases, the leaf may have added to it definite kinds of tissue for storing water to be used in time of need.

(2) That light readily causes various reactions in many of our ordinary plants, every one knows. Watch the folding of the

PLATE No. 4.

PLATE No. 5.

Clover leaves as daylight decreases, and you will see them go into their sleeping position, while in the morning they open their faces and present them to the sky. Look at the Wistaria vine by the aid of a lamp, late at night, and you will see that the little leaflets have dropped down and closed together. If the morning is bright and warm, they will rise early and by nine o'clock every young tender leaflet will point its tip directly at the sun, in a direction parallel with both the light and heat rays. As the heat of the sun increases toward noon and during

the hotter part of the day, you will find these leaflets constantly rotating, maintaining their parallelism to the sun's rays. If the day is cloudy and the heat of the sun obscured, these same movable sensitive leaflets may remain in a stationary position so as to receive the most direct light possible during the whole day. If you choose to wander in your nearest woods, many similarly-acting plants cannot fail to escape your notice, such

PLATE NO. 6.

PLATE NO. 7.

as the *Desmodium*, the *Lespedeza*, the *Melilotus*, and, among the tree-like forms, *Amorpha*, and *Robinia*, or the Locust.

Plate No. 4 shows the *Melilotus*, or Sweet Clover, on a cool, moist, somewhat cloudy morning, when the leaves are spread out fully, striving to receive all the light and warmth possible. The direction of the light is generally at right angles to the surface of the foliage.

Plate No. 5 represents another plant on an extremely hot, dry morning in midsummer, photographed at nine o'clock.

The photograph was taken from the south. It will be seen that the three leaflets, elevated on their sensitive petioles, are all pointing directly at the sun in the south-east. The leaflets being parallel to the heat and light rays, escape much of the consequent transpiration of water which would occur were they thrown down to the sun's rays as in Plate No. 4.

Another plant, Plate No. 6, photographed under similar conditions of dryness and hot sun at twelve o'clock in the morning, shows the leaves pointing directly to the zenith, thus again parallel to the sun's rays and for similar reasons.

PLATE NO. 8.

Plate No. 7 shows still another plant taken on the same hot and dry day, with the leaves pointing directly toward the sun. This photograph was taken at 2 P. M. in the afternoon from a position 15° north of west, at right angles to a profile view of the leaves.

It will be seen from these illustrations that when the conditions are ripe for rapid loss of moisture, that these sensitive leaves continually keep themselves parallel with the sun's rays during the day. As the leaves in the morning are first influenced by heat and light, they elevate themselves and point toward the eastern sun. They continue to rotate during the day, so that at noon they are erect, slightly inclined to the south, and at night are directed to the west.

Plate No. 8 shows a cluster of these plants photographed from the north at six o'clock in the afternoon after a comparatively warm, dry day. The leaves are mainly pointing to the

right toward the western sun which is still quite high. At sundown, after the influence of the falling dew has been slightly felt, the leaves will begin to assume the position seen in Plate No. 4.

If we continue to watch this plant as darkness comes on, we shall find all these leaflets dropping down slightly, each turning itself one-fourth around on its petiole until the three present their edges uppermost. The two outer leaflets, having their faces toward each other, move slightly toward the central one until they touch it. It will be seen that the three little leaflets have thus placed themselves together in such a way as to reduce their surface to nearly one-third of the original area. This protects them from radiation and the loss of much heat during the night. Plate No. 9 shows a plant while sleeping, the photograph having been taken at twelve o'clock midnight.

Plate No. 9.

But there are other effects of light more marked than these reactionary movements. In ordinary plant assimilation, which takes place only under the influence of light, the leaves are generally the active organs. It sometimes happens that when the light becomes insufficient for assimilative purposes, certain parts of the organ itself — the leaf — become quickly atrophied or disappear entirely. Thus the form of the elm leaf has gradually become oblique on account of the shading of one

of its sides. This is true, too, of the house begonia and many others of our common plants.

At high altitudes, where the differences of light and shade are much more intense, these changes are correspondingly more quickly brought about. With these external changes of form there are microscopic differences which are even more striking. In Plate No. 10 are shown two cross-sections of leaves taken from the Mountain Balsam (*Abies fraseri*), *a* from a sunny exposure and *b* from the more densely shaded part of the tree. In Fig. *a* we have on the upper part of the leaf the palisade cells above, and the more loosely arranged tissues,

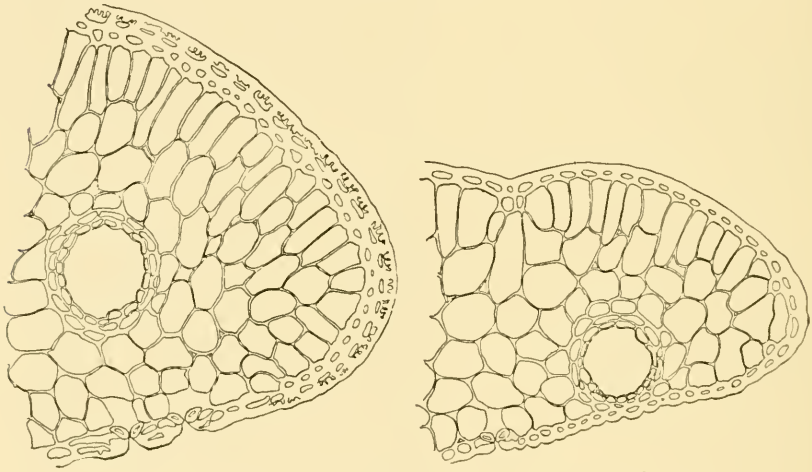


FIG. *a*. FIG. *b*.

PLATE NO. 10.

for assimilation, below. About the middle of the leaf is a large resin duct. Fig. *b* is the cross-section of a similar leaf from the same tree, carefully selected from a point underneath the heavy branches which the direct sunlight never reached. Above the resin duct, on the upper side of the leaf, are the palisade cells, much as in *a* ; but the loosely arranged assimilating tissue beneath the resin duct is seen to be absent in *b*. In other words, the light was not strong enough in the lower shaded parts of this tree for assimilative purposes, and in consequence these tissues have been dispensed with.

(3) And here let me speak of the influence of altitude, which affects not only the light but also the temperature and the air

supply. It will be readily understood that at any very high altitude the amount of dust in the air is at a minimum; also, that the greater the altitude the less dense the atmosphere, which will, consequently, contain less moisture. But as the illumination of general space depends wholly upon the refraction of light from the dust and particles of moisture in the air, it will be seen that refraction at high altitudes must be at a minimum, and that air spaces outside of the direct line of the rays of light will be much darker than at lower altitudes. The light given to shaded leaves, then, at these high altitudes through refraction, often proves too little for the purposes of assimilation, and the assimilating tissue consequently disappears.

This shows the relation of altitude to light and assimilation. But altitude is many-sided in its influences. Because we have here less dust and less moisture in the air, the sun's rays, not being refracted out of their course, are much more direct. In the shade, at an elevation of seven thousand feet, you are too cool, but step into the sunlight and your upturned face will be quickly burned by its strong, direct rays. These same rays burn the moisture rapidly out of mountain leaves. To avoid this as much as possible, they become thickened in many ways, often decrease in size, and travel along the same lines generally for water retention as have the desert plants. High altitudes, then, will produce desert-like plants, as seen in the Live-for-evers, or *Sedums*. Not only are the leaves found decreasing in size and becoming thickened, but in all sensitive plants the movements are much more rapid.

Many plants which do not have daily movements of leaves to accommodate themselves to the light, still put their young leaves, when thoroughly exposed to the sun, parallel to its rays in the hottest part of the day. Later in the season, when the epidermal tissues are more thoroughly developed and the cuticle has been fully thickened, the leaves, thus becoming better able to resist the heat rays, drop down to a position more or less at right angles to the approaching light. Select another plant of the same species, which is thoroughly shaded during the whole day — it may not be twenty feet from the first — and we shall find that the young leaves, as they develop themselves, at once

drop down to a position in which the approaching light will be at right angles to their surfaces. Plate No. 11 is a photograph of *Rhododendron maximum* taken at an altitude of over 4000 feet. It developed itself entirely in the sunshine. It shows the upper, or young leaves, erected vertically upward until they are nearly parallel with the sun's rays during the hotter portion of the day. The leaves below, which are more or less horizontal, are more than one year old. At the end of the

PLATE NO. 11.

first year, the upper leaves, having become tough and coriaceous, will assume a position parallel to the older ones below.

In Plate No. 12 is shown a photograph of *Rhododendron catawbiense* at the same altitude, but standing in a thoroughly shaded spot. Although a different species, the action of the leaves is the same. It will be seen that the young and growing leaves above put themselves quickly in a position parallel with the leaves below during development.

The differences between the microscopic structures of the leaves exposed to direct sunlight and of those wholly shaded

are even more marked than the corresponding differences in position. Plate No. 13 shows two sections of leaves from *Rhododendron maximum*, the one from the sunlight, the other from the shade. Section *a*, from the sun, shows four superimposed rows of palisade cells, extremely long and narrow, arranged with their long diameters pointing to the surface of the leaf in a way to afford the best protection from the heat rays. Section *b*, from the shade, has no very well marked palisade system, for no such protection is needed. The section

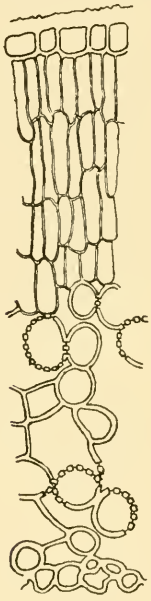
PLATE NO. 12.

from the sun is much thicker than that from the shade, but while the loosely arranged mesophyll in the under part of the leaf, where assimilation is most active, occupies in the sun section only one-half of the space of the whole leaf, yet in the shade section it includes more than two-thirds. The explanation is simple. The exposed leaf requires less space for the work of assimilation on account of the strong light. The shaded leaf, on the contrary, as much less light reaches it, must have a greater number of cells in which to do a given amount of work.

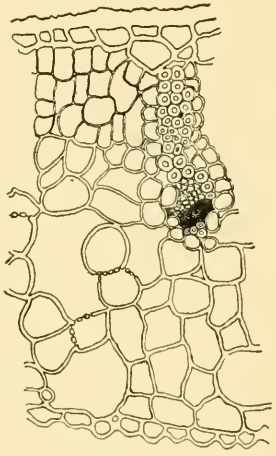
(4) The food supply of the plant may exert a very strong influence upon its development and life. It is a well-known

fact among gardeners that over-fed plants will often produce no fruit. When pear-trees refuse to bear fruit, they can often be made prolific by cutting away the central root, thus lessening the supply of nourishment. As a rule, when plants are poorly fed, they put forth blossoms and ripen their fruit earlier, although it may be smaller and of poorer quality.

(5) The influence of the sea upon plants is quite remarkable. We have seen that in desert regions, and to some extent in high altitudes, plants have greater or less tendencies to take on consolidated forms. This is also true of seashore plants.



SECTION *a*.



SECTION *b*.

PLATE NO. 13.

The cause in both desert and mountain regions was a lack of moisture. On the seashore, at the water's edge, and even with many plants standing in the water, we find these thickened leaves and leafless forms. Hence we must look for another cause for this than lack of water.

Ordinary land plants secure the water which they need mostly through their roots. Although the soil may be apparently dry, yet the plant has no difficulty in supplying its necessities, for every particle of the soil is surrounded by a

film of water, which is forcibly taken from it by the osmotic action of the root hairs. Each hair is an elongation of a single epidermal cell. Its external wall is composed of porous cellulose. Besides the ordinary cell contents, such as nucleus, masses of living protoplasm, vacuoles, microsomata, and the like, there is a thin semi-fluid layer of protoplasm, closely appressed to the whole interior of this porous cellulose wall. This forms, with its cellulose support, an osmometer, and when the root hair is tightly crowded in its growth against the surrounding particles of earth, thus coming in contact with the water film, there is at once set up, with great force, a current from the thin fluid surrounding the particles through the walls to the more or less thickened one within. Under a pressure often of nearly an atmosphere, this water is forced from the exterior cells of the root to the interior conducting fibro-vascular bundles, through which it finds its way to every part of the plant. It will be seen that the condition of this forcible absorption is the interposition of a protoplasmic membrane between two fluids of different densities, the more dense, toward which the current always tends, being within the cell. It is only necessary to state that in sea-water we have a fluid of even greater density than is usually found within the cells of plants, and that this would tend at once to make it either very difficult or utterly impossible for the plant to secure its water. Plants which have adapted themselves to the influence of sea-water have done it in a number of ways — sometimes by taking large quantities of salt into the cells, which balances much of the salt without. In this way the density of the fluid within is made greater than that of the water without, and absorption takes place to a limited extent. But since it is still very difficult for the plant to secure a large amount of water, although it may stand in it, — "Water, water everywhere, and not a drop to drink," like Coleridge's shipwrecked mariner, — all the other methods of retaining its scanty supply, usually found in desert plants, are here also exemplified. Thus, the leaves may become smaller or thickened or disappear altogether, in order to lessen the transpiring surface. If the

plant can get but little moisture, it also retains that little, or lets it go with great difficulty. Hence, we have on the sea-shore a vegetation corresponding in some respects to that in high altitudes, or to that in desert regions, although the apparent conditions are radically different from either.

Hydrocotyle umbellata is a plant common in moist or watery places from Maine to Florida. In the semi-tropical fresh-water marshes of Florida, it places its slightly thickened, rounded leaves at right angles to the sun's rays, parallel to the surface of the water in which it grows. When it happens to encroach upon the salt-water marshes of this same region the difficulty of water supply is so heightened that the slender petioles all make a right-angled turn at their upper end and put

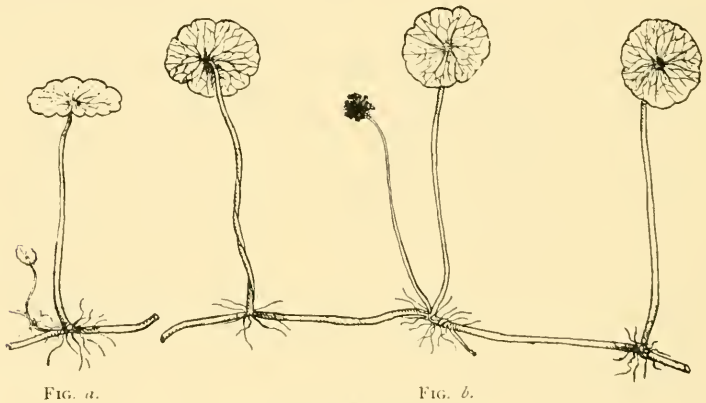


Fig. *a*. Fig. *b*.

Plate No. 14.

the now thickened leaves in a vertical position, in order to avoid the direct rays of the sun, thus lessening the loss of water.

Plate No. 14. fig. *a*, shows a sketch of this little plant, taken from a fresh-water stream in South Florida. Fig. *b* on the same plate shows another plant taken from a salt marsh near by. All of its leaves are rendered vertical by this turn in their petioles. The microscopic differences are much greater than this relative difference in position. The leaves of the one in the salt marsh have become much thicker, the epidermal tissues much heavier, the number of the palisade cells has increased, the intercellular spaces have nearly disappeared, and the

openings into the stomata have been transformed, through thickening of the cuticle, into narrower, deeper channels. These differences are common to many other plants which inhabit indifferently fresh and salt water locations.

The plants which grow many miles from the sea-shore are also more or less influenced by the salt in the spray which is mechanically lifted by the winds and carried long distances inland. Lighting on the leaves, it has a tendency to draw out the moisture from within and in this way very materially increases the normal transpiration. To guard against this many plants near the sea-shore thicken up both the cuticular and epidermal layers. Where the wind blows somewhat constantly from the sea over the land, these effects may be seen in plants extending from the shore sometimes as far as twenty miles.

Many strand plants exhibit the same peculiarities as those of the Rhododendrons of the mountains, *i. e.*, they erect their leaves parallel with the rays of the mid-day sun to avoid loss of moisture. This is especially marked in the Mangroves of our Southern coast, which, for a greater part of the day, stand directly in the water. The plants on our New Jersey shore illustrate many of these facts and are quite as interesting for study as those of South Florida. A half-dozen plants of one of our pigweeds (*Atriplex littoralis*) taken from a salt marsh, potted, and carried to a greenhouse in the city, exhibited the following interesting characteristics: On the salt marshes from which they were taken, all the leaves were rigidly erect, making no shadow with the mid-day sun. But after being watered with fresh water for five or six days in the greenhouse, all of the leaves dropped down to a normal position, presenting their faces to the direct rays of the sun. Upon watering them for several days following with a strong salt solution, the leaves again erected themselves, assuming the precise position in which they had grown on the salt marshes.

It has long been known, through physiological experimentation, that almost any salt or alkaline solution decreases transpiration, because it at once lessens the water supply. The converse is true of acid solutions. This law may be taken as

a partial explanation of the conduct of the little New Jersey Pigweed.

Perhaps enough has been already said to convince anyone that the almost infinite variety of plant forms may have some fairly direct connection with their environment. The cypress tree produces its needful aerating organs when grown in the water, but no sign of them in dry soil. It may be a matter of wonderment, however, why these characteristic organs are so quickly and readily lost. They are an acquired character to fill a definite want — when this want no longer exists, they disappear. Perhaps this character, which we know was acquired since the Eocene period, has endured for too short a time for permanent inheritance. Many other plant characteristics acquired on physiological grounds, but regarding the duration of which we have no record, although we have reason to believe that they have been serving their purpose for an immense period of time, are perfectly permanent, and inherited by their offspring long after the developing cause has disappeared. We have in Australia a large number of trees which have adapted themselves to a hot and dry climate by various manipulations of the leaves. In some Acacias, for example, the leaf-blade has entirely disappeared, and the wing growth on the upper and under sides of the petiole is an apparently vertical leaf. Other members of the same family make the true leaf vertical by a twist of the petiole. Although accomplished in two very different ways, the object in each case is plainly to lessen the tranpiration. Apparently, this is a much more trivial change than the acquirement of knees by the Cypress. Both fulfil a physiological necessity ; but while the knees of the Cypress are lost in succeeding generations if the developing cause is removed, the Acacias may be cultivated for generation after generation, entirely removed from the climatic conditions which produced the changes, yet they will be as permanently inherited as in their own native land.

Avicennia nitida, the so-called Black Mangrove of our Florida and West Indies coast, of which I have already spoken, belongs to the Verbena family. Normally a dry land form, it

has adapted itself to a growth between tide waters. In so doing it not only produces the remarkable, vertical, negatively geotropic roots, which grow up out of the salt mud, so that at low tide they remain each day several hours in the air for oxygen absorption, without which it could not live, but it has also remarkably thickened its leaves and appressed them vertically against the stems in order to protect itself from loss of water, for reasons which have been already stated.

From the history and general relation of this mangroove to the Verbena family, we judge that this adaptation is of comparatively recent date. When removed to dry cultivation it loses all these characteristics in the first offspring. In the case of the Cypress we have not merely theoretical reasoning from family relationship, but also definite data from fossil remains, which prove that it was a dry land form in a very recent geologic age, and that its aquatic habits are modern. It is certainly curious, and you may interpret it as you please, that both these plants, when subjected to opposite conditions, immediately lose their acquired characters. In the Australian plants, on the contrary, similarly acquired characteristics under opposite conditions are perfectly permanent. That they have been in this condition for an immensely longer period of time, and that in consequence this development is so stamped upon their parent stock that it reappears generation after generation, even when withdrawn from their causal surroundings, is, perhaps, the key to this riddle.

Let me, then, remind you once more of the almost infinite variety and diversity of plant forms, and that, as seen in nature, we can generally trace the relationship between the peculiarities of the plant and its surroundings; also, that in general, these peculiarities, no matter what they may be, reproduce themselves in the offspring. Let me state once more that any plant which we may select as an example, no matter how peculiar in form or habit of growth, gives us an expression in this peculiar form and special habit of the forces which have surrounded it and touched it all the way down its long line of descent, from its earliest more simple and primary condition to its present more complete expression of plant life.

NINTH LECTURE.

IRRITO-CONTRACTILITY IN PLANTS.

PROF. J. MUIRHEAD MACFARLANE, D. Sc.

IN a paper published by me about nine months ago, I showed that the generally accepted view that the leaf of *Dionæa* contracted after one stimulus of the irritable hairs was incorrect, and that two stimuli were necessary to cause contraction. The other phenomena connected with leaf-closure, and described in the paper, were so remarkable as to cause me to inquire whether these phenomena were unique in the vegetable kingdom, or whether conditions could be traced that connected *Dionæa* with other sensitive plants.

The behavior of the leaf of *Dionæa* to mechanical stimuli given at varying intervals of time was such as to suggest a very definite and exact contraction of the protoplasm of certain cells. The outcome of the inquiries which are epitomized in the present lecture, and which I hope in time to bring forward in extended form, proves that in the vegetable as in the animal kingdom, we have to do with a true contractile tissue. Further, among those higher plants that we are now to study, this tissue is made up of cells, each consisting of an irrito-contractile protoplasmic sac enclosing a quantity of sap, and each cell is joined to neighboring cells by protoplasmic processes that pass through minute pores in the common cellulose membranes.

In order to make clear the relation that is shown by many sensitive leaves to environmental stimuli, it may not be inappropriate to indicate first the possible movements that such leaves can perform, as generally stated in memoirs and text-books on the subject. A plant of the common yellow sorrel

(*Oxalis stricta*), if examined on a quiet and moderately cool day when the temperature is 25° C., will show the trifoliate leaf fully expanded, and the three leaflets so placed on their stalks that their surfaces lie at right angles to the sun's rays; *i.e.*, they are not only heliotropic in that they grow toward the light, but they are diaheliotropic in that they can, if need be, so place their surfaces to the light as to receive the rays at right angles. But if the temperature rise to 29°–30° C. in the shade, the leaflets will begin to fall down; and if a steady increase in temperature goes on, they will continue to fall till at 33° C. they will incline downwards back to back. This day-movement has long been known to occur in many plants, and was regarded as a means by which the leaves were screened from intense illumination; but my colleague, Prof. W. P. Wilson, considers it to be a heat-movement, or an attempt on the part of the plant to screen itself from intense solar thermal action. As connected with the varying results of stimulation on different species afterward to be described, it may here be noted that *Oxalis stricta* shows distinct heat-movement at 29° C.; *Oxalis Deppei* at 31° C.; *Oxalis dendroides* at 33°–34° C.; while the common sensitive plant (*Mimosa pudica*) that has thin and apparently delicate leaflets only becomes affected when the shade temperature rises to 37° C. This movement, described by Darwin as a paraheliotropic one, I propose to term 'parathermotropic.'

But as the sun's rays become tempered in the afternoon, or as plants formerly exposed to the full heat of the rays get sheltered by foliage, the flat, expanded condition is resumed. Toward the approach of evening the leaflets again begin to fall, and by 8.15 or 8.30 during midsummer have taken up the same position that they had during the heat of the day. This night-sleep, or nyctitropism, has, with every show of reason, been viewed by Darwin and others as a protection against too rapid radiation of heat and reduction of temperature in the tissues. We would emphasize it, then, that alike during the parathermotropic and nyctitropic states the leaflets of *Oxalis stricta* are similarly placed. The nyctitropic position is retained till the following morning, and in some plants, at

least, we have evidence for believing that the effects of it are not fully overcome till 7 or 7.30 A.M., in June or July.

The movements now described are characteristic of a large series of plants belonging to many natural orders, but the group *Oxalideæ* of the order *Geraniaceæ*, and the order *Leguminosæ* probably include between them about three-fourths of the entire number.

You are doubtless all aware, however, that leaves or leaf-parts may exhibit movements which serve a different purpose in the economy of the plants that bear them. The leaves of *Dionæa* and *Drosera*, as well as tendriliform leaves and leaflets, are examples in point; and the question naturally presents itself, — How are these movements effected? Before attempting an answer, we propose to lay before you the results of observations and experiments which may aid in the solution of the question.

Naturally, in the vegetable as in the animal world, irrito-contractility can only be started by stimuli of a mechanical, chemical, thermal, luminous, or electrical nature. For several reasons, I can only treat briefly here the effect of the first three forms of energy, and I will select plants for illustration in the order that seems most suited for elucidating the subject. First, we may recapitulate regarding *Dionæa*. As my published researches show,[1] a summation of two mechanical stimuli is ordinarily necessary to start contraction. Further, these must be applied with a time interval between of at least $\frac{1}{4}$ second; for if two stimuli are given in rapid succession, both are propagated through the protoplasm as one wave, so far, at least, as motion of contraction is the visible outcome. But though one stimulus or two rapidly applied stimuli are insufficient to start contraction of the leaf-halves, we know that active molecular changes are in process, for the leaf-halves exhibit delicate but visible wave impulses passing along them from base to apex, while Burdon Sanderson has demonstrated that active electrical changes are going on. After a second mechanical stimulus, or three, if two are rapidly applied, the leaf closes partially, — *i. e.*, the marginal bristles loosely interlock. If

[1] Bot. Cont. Univ. Penn., Vol. I, No. 1, 1892.

the leaf be not further irritated by some instrument, or by a caught animal, it slowly relaxes after 12–15 hours; but if additional stimuli are given, the leaf gradually and firmly tightens up till its margins become recurved. Prolonged stimuli, either of a mechanical, chemical, or electrical nature, start eventually (after 8–10 hours usually) the flow of an acid secretion from glands that cover both halves of the leaf. It is clear, therefore, that complete contraction of *Dionæa* leaf can only be effected by a summation of stimuli; and such stimuli may either be partially or entirely mechanical, chemical, or electrical. I have further shown, contrary to prevailing opinions, that not merely the three hairs of each leaf-half, but the entire surface is irrito-contractile. Thus, a minute bit of ice or a drop of hot water placed on any part of the leaf away from the hairs, also a forceps' pinch, a mechanical shock, and many chemical agents, excite to closure.

We have already said that the time-interval between two shocks may be too short to start contraction, but it is equally true that too great a time interval is attended with no visible change; in other words, the piling on of the second stimulus to the first, if too long delayed, is insufficient to effect closure. With an interval between of 50–60 seconds, at a temperature of 14°–21° C., contraction takes place after a second excitation; and I stated that if the interval were increased to 90–120 seconds, the strength of the two was almost entirely cancelled. Through the kindness, however, of my friend Mr. Aldrich Pennock, I have studied plants in his hot-houses at high temperatures (35°–40° C.), and find that the effects of previous stimuli can be retained for 4 minutes at least. But a second stimulus applied 4 minutes after the first causes no visible movement of closure; a third, 4 minutes after the second is similar; and not till the sixth stimulus does an almost inappreciable contraction of the halves take place. Summation of successive stimuli from the eighth to the twelfth gives added force — sufficient to bring the halves together. Here, then, is an irritable tissue that steadily contracts, or prepares for contraction, through a period of from 30–45 minutes.

The conditions thus revealed by *Dionœa* were so arresting as to cause me to inquire whether similar phenomena might not be demonstrated in other sensitive plants. Botanists have long been acquainted with certain species which from their contractile movements were aptly designated "sensitive plants." These included species of *Oxalis*, *Averrhoa*, *Mimosa*, *Cassia*, *Schrankia*, etc., some of which were considered to be highly irritable, others less so, and others, again, scarcely deserving the name. The best account hitherto given of the action of some of these when affected by mechanical stimuli is to be found in Pfeffer's *Pflanzenphysiologie* (pp. 224–254), where *Mimosa pudica* and *Oxalis acetosella* are chiefly dealt with.

Now I hope not only to show you that various transition types exist between such roughly classified groups as are expressed in the descriptions "very sensitive," "slightly sensitive," "scarcely sensitive," but that under given environmental conditions all exhibit a definite irrito-contractility, characterized by varying degrees of latent period, of contraction period, of expansion period, and of summation effects, or, to put it in a broader sense, that *the same phenomena of irrito-contractility are encountered in the vegetable as in the animal kingdom.* The optimum temperature for most of the plants now to be mentioned is an average of 26° C, exposure for a short time or for a prolonged period to low temperatures (8°–15° C) causing an extension of the latent period, slower rate of contraction, and a reduced power of conducting stimuli.

As being both a common plant and a central type in its physiological behavior, we may now take the field and wayside weed *Oxalis stricta*, the yellow sorrel. When plants are grown in a rather shady situation and exposed to a temperature of 18°–24° C., the leaves have a rich green color, and the three leaflets together form a triradiate rosette. We need not now refer to structural details further than to say that at the base of each leaflet is a little cushion composed chiefly of small, densely aggregated, and vacuolated cells forming a typical sensitive pulvinus. After a sharp but delicate mechanical stimulus applied with a pencil or other instrument to a terminal

leaflet, a latent period of $3\frac{1}{8}$ seconds elapses, followed by a period of slow but gradually accelerating contraction during the next 4 seconds. From the seventh to the twentieth second the motion is rapid, but thereafter slows down gradually to the thirtieth second, and then becomes increasingly slow till the forty-fifth second when the contraction ceases. After 15–18 minutes expansion begins, and a very slow rise can be traced till the leaf regains its expanded state in 45–50 minutes. The angle through which such a leaf falls is on the average 37°, but varies with the daily periodicity of cell tension, as well as the temperature and moisture of the air and soil.

It should be stated that young, active — not necessarily growing — leaves are to be preferred for experiment, but even the oldest and fully matured leaves are sensitive to a definite degree, though less so than those younger. Some interesting statistics will be adduced later in this connection.

When the three leaflets of a leaf are simultaneously excited, all contract, and the movement shown is as follows: The terminal leaflet simply drops and folds its halves together more or less toward their upper faces, but the two side leaflets move forward and downward so that each describes a segment of an ellipse. If care be taken, however, to stimulate the terminal one of the three, it alone will contract, or, at most, the side leaflets will move to a small extent. This, among other things, proves that the capability of conducting a stimulus through its tissues is comparatively feeble in this species.

In no case have I found that a single shock is sufficient to produce a contraction approaching in amount to the nyctitropic or the most pronounced parathermotropic states. The angle fallen through varies from 25° to 48°. A summation of stimuli, however, produces very different results. If an excited leaflet be left till the downward movement has ceased, and a second stimulus be applied, it will fall still further, but through a less angle than before, and its motion will cease sooner ; if a third stimulus be then applied there will be an additional fall less in extent than the second, and greatly less than the first, the time of motion being correspondingly shortened. A fourth stimulus will give a slight but appreciable

additional contraction. I may best illustrate by a concrete example selected at random from many others. A leaflet stimulated fell through 42° in 45 seconds, a second stimulus increased the angle to 69° within 38 seconds after rest, a third stimulus increased it to 81° after 33 seconds, and a fourth to 84° after 27 seconds.

Thus, by four successive stimuli applied during a period of 143 seconds, the leaf described an angular movement of 84°. But, as I have already stated, the period of maximal movement after first excitation occurs between the seventh and twentieth seconds. Taking advantage of this fact, by shortening the time interval between the stimuli, the same amount of contraction can be got in a much shorter time. A leaf was irritated and allowed to contract for 22 seconds, when it had fallen through 38° ; it was again irritated, and allowed to contract for 20 seconds when it had fallen through 61°. Again irritated it fell through an additional 15°, and after the fourth stimulus had fallen through 81°.

The above time-intervals remain wonderfully constant in all active leaves when the environmental conditions remain constant, but a distinct shortening of the latent period by $\frac{3}{5}$ to $\frac{1}{2}$ second was noted on a moist, close and warm morning in early July after a thunder-storm of the previous evening. It should also be stated that when the plants are continuously exposed to such high shade temperatures as 35°–40° C., they become smaller in size and irregular in action.

We now turn to thermal stimuli, and I may at once state that ice particles produce a very marked effect on this and all other sensitive plants. When a piece weighing $\frac{1}{4}-\frac{1}{3}$ grain is placed at the junction of three leaflets a longer latent period than for mechanical stimuli ensues, but when once started contraction steadily proceeds till the ice has melted and the resulting water has attained a temperature that fails to excite the protoplasm. If by aid of a pipette or blotting paper the water is sipped off and a fresh bit of ice is placed, contraction will go on till the nyctitropic position has been reached. But as in the case of mechanical stimuli more localized action can be started, for if ice be placed not on the pulvini but near

the base of the terminal leaflet, the latter will move through a large angle, while the side leaflets will not at all or only slightly participate. A bit laid similarly on one of the side leaflets will make it move independently of the others. By conducting control experiments with weights equal to the particles of ice used, it can be proved that cold stimulus and not weight of the particles is the determining factor.

As with *Dionæa*, so here a drop of hot water and the application of a heated wire excite to contraction, but instead of touching analytically on these I should prefer to dwell on the question of parathermotropism. All my observations go to show that this is largely due to heat stimulation of the irrito-contractile cells, acting steadily for some minutes on these, and that the movements can be hastened or arrested by a rise or fall of a few degrees of temperature. Moreover, it seems undoubted that the soil temperature has much to do with the amount of contraction that each leaf undergoes. It is not difficult to understand why this should be. Given a soil on which *Oxalis* is growing that is covered by a pretty close herbage, above which the plant rears its tuft of leaves; the soil protected from the sun's rays, and retaining its moisture by reason of the roots that spread through it, will furnish currents of cool water that will constantly rise into the leaves of the plant. Evaporation of the surplus moisture thus passed into the leaves will further lessen the temperature of the tissues. I may be allowed to quote only one set of statistics in support. On a close, dull, but rather warm day, with the temperature at 25° C. in the shade, several plants were studied that grew under the shade of trees, and amid an abundant herbage. The ground temperature was 23° C. All the leaflets were either fully expanded or inclined slightly upwards from their point of union. Another set grew on a rather moist bank and were slightly overshadowed by a tree. The thermometer placed alongside the plants, and like them exposed to a slightly higher than the true shade heat, registered 27° C., while the surface of the soil below registered 25.5° C. The leaflets were flat or faintly inclined downwards. Alongside a third set, about two yards from the last, the thermometer

registered 29.5° C. when exposed, and 27.5° C. on the ground. Some plants that were exposed to the sun's heat grew on a rather dry soil with scant herbage, and their leaflets were deflected through an angle of 52° to 65°. Lastly, on a bit of hard, dry, whitish soil, the exposed temperature alongside the plants was 31° C., and on the soil 33° C. The leaflets of the plants were as strongly deflected as during night-sleep.

The amount of moisture, therefore, that is drawn from a cool shaded soil or from a hot, dry soil may largely determine, by its temperature, the stimulus given to the protoplasm and the position that the leaflets are to assume accordingly. A coöperating factor in the parathermotropic movement may be a lack of sufficient moisture, which, in all sensitive plants studied, causes flaccidity and a want of tone in the tissues.

We shall have occasion in treating of other plants to speak of the action of such chemicals as ammonia, carbonate of ammonia, chloroform, ether, alcohol, etc., which all act as excitants.

Oxalis Deppei is a large succulent species, commonly grown now as an edging plant in herbaceous borders. Each leaf is quadrifid, and examination of the base of the leaflets reveals large reddish swellings or pulvini. Mechanical stimulus is followed by a latent period of $3\frac{1}{8}$ seconds; then slow but accelerating contraction is observed for the next 4 seconds, when rapid contraction follows for 22–23 seconds, and a very gradual slowing down goes on till contraction ceases 80–90 seconds after stimulation. The striking and main difference between this species and *Oxalis stricta* consists in the contraction period being about twice as prolonged. As with *Oxalis stricta*, summation series can be obtained that vary with the time-intervals between stimuli and the environmental surroundings.

Observation of the contraction and expansion movements of this and other sensitive plants shows that each is made up of numerous minor contraction and expansion waves that cause the leaf to move by minute jerks, each minor contraction phase during the great period of contraction being much greater in amount than the succeeding expansion phase. In all prob-

ability this is due to the gradual passage of sap through the contractile protoplasmic layer of each cell and the elastic recoil of it and of the wall as additional liquid is extruded or absorbed.

I now pass to *Oxalis dendroides*, a plant eminently suited for investigations like the present, and which has yielded results as interesting as they were unexpected. For a supply of fine specimens I am indebted to the kindness of Mr. G. Oliver of the Washington Botanic Garden. It is an abundant weed in Brazil and is often confounded with *Oxalis sensitiva*, that is native from Persia to China. It is one of a series of nearly related forms, some of which like the present have a simple unbranched upright habit, while others incline to a proliferous mode of growth. Though seldom seen outside botanic gardens, it grows with the utmost readiness, fruits freely, and scatters its seeds widely. Four noteworthy points can be readily demonstrated on this species, though witnessed less perfectly in others. These are: First, that the latent period varies according to the age of the leaf; second, that a gradual propagation of stimulus from base to apex or *vice versa* can be shown to exist; third, that the rate of propagation of the contraction-stimulus can be exactly measured; and fourth, that the rate of contraction and expansion of leaflets is quickest in young and slowest in old leaves.

If the tip of the blade of a terminal leaflet be stimulated by a forceps snip, in an atmosphere whose temperature is 28°–32° C., it and the companion leaflet will fall down through an angle of 40°–45° in about 20 seconds. That irritation of a leaflet should excite to a rapid movement not merely of one but of neighboring leaflets, suggests the idea *that at least some of the cells of every leaflet can conduct a stimulus*. This idea is entirely confirmed by many other experimental results. If the leaflet that has been irritated be part of an old leaf, the succeeding pairs of leaflets will close in regular succession from apex to base with a time interval between each pair of $2\frac{1}{2}$ seconds. By this we mean the time required for propagation of the shock from one pair of pulvini to another pair below, and succeeding contraction of the living protoplasm of the different cells in the path of the stimulus.

But as I shall point out later, it would be a mistake to suppose that the actual rate of propagation of stimulus from apex to base of the leaf is so slow as this. If the leaf operated on was the fifteenth from the growing apex of the stem and the tenth leaf be now chosen and similarly operated on, the time interval between contraction of succeeding pairs of leaflets will be $1\frac{7}{8}$–2 seconds; with the seventh leaf from the apex the interval will be $1\frac{1}{2}$–$1\frac{5}{8}$ seconds; and finally a delicate light-green leaf, such as the second or third from the apex, shows closure of successive pairs with an interval between of 1–$1\frac{1}{8}$ seconds. The latent period shown after general stimulus in such a set of leaves is equally variable, and under optimum surroundings is only $\frac{1}{2}$–$\frac{5}{8}$ of a second in young leaves such as the third from the apex, $\frac{3}{4}$–1 second in the seventh or eighth, 1–$1\frac{1}{4}$ in the tenth or eleventh, and $1\frac{1}{2}$–$1\frac{3}{4}$ in the fifteenth.

As mentioned above, the angle through which the leaflets fall on excitation, is from 40°–45°. If a general shock be given to the entire leaf, the leaflets fall simultaneously through an angle of equal amplitude. But summation action can now be brought to bear, for on second stimulus the leaflets will fall through an angle of 24°–28° and in a correspondingly short time. On third stimulus the leaflets will fall through 12°–14° in a still shorter interval, while a fourth stimulus will cause a fall through 4°–5° in a shorter interval than the last. By this time the leaflets will be folded downward back to back as in night-sleep, or a fifth stimulus effecting a slight additional movement may be needed to complete the process.

A small bit of ice weighing $\frac{1}{3}$ of a grain, if delicately placed on the tip of a terminal leaflet so as not to touch or directly stimulate the pulvinus cells, starts motion in the one opposite within 7 seconds. A steady impulse is then propagated down the leaf-stalk from pair to pair, the time interval, as in mechanically stimulated leaves, depending on the age of the leaf. If, as has repeatedly been noticed, a pair of leaflets is encountered which is somewhat benumbed from the effect of previously applied chemical or other agents, these will remain motionless, or nearly so, but the same interval of time will elapse before the next pair beneath will contract as would have been

needed to start movement in the benumbed ones as well as in these. On several occasions, a behavior has been noted, both in *Oxalis dendroides* and the common sensitive plant, that is worth recording, whether the suggested explanation of the behavior is the correct one or not. A bit of ice laid directly on the pulvini of two opposite leaflets failed to excite them to motion and even a pair beneath would only partially contract, while those still further down would move in normal fashion. I satisfied myself that the position and weight of the ice particle offered no obstacle, and learned also that if within 20–30 seconds the ice be removed, the leaflets after an added interval of 7–15 seconds fall backwards as if recently stimulated. We believe a probable explanation here to be that during the latent period of excitation, the ice had so lowered the temperature of the subjacent cells as to benumb the protoplasm, which only regained its contractile properties with returning irritability after removal of the ice.

We may next inquire whether a peripheral or centripetal stimulus is propagated to the leaf base or to the stem more rapidly than a centrifugal stimulus initiated at the basal leaflets. The only plant that has hitherto been experimented on is the common sensitive plant, but Dutrochet and Bert expressed different views. *Oxalis* is much more convenient for the determination of this question. Each leaf carries 15–24 pairs of leaflets. A particle of ice placed on the pulvini of the middle pair, *i.e.*, the tenth if there are 19 pairs, will excite all the pairs above it within 15–17 seconds, but 21–23 seconds will elapse before the lowest pair in such a leaf as the tenth from the apex-bud closes. With the ice in varying positions and on leaves of different age I have invariably found that a centrifugal is more rapid than a centripetal impulse, and I have studied many examples. But the records are less satisfactory as regards simultaneous basal and apical initiation of excitation, though the balance of experimental proof is in favor of centrifugal stimulus.

As with sensitive plants in general, carbonate of ammonia is a powerful stimulant, and its rapidity is proportioned to the strength of the solution. When a small drop of a 20 %

solution is laid on the tip of a terminal leaflet, striking changes occur. Both of the terminal leaflets close within 7 seconds, and the succeeding pairs below begin to close at the rate already given. But before 5 or 6 pairs out of, it may be, 19–22 have closed, a leaflet near the base may be noticed to twitch, or, as often happens, leaflets on distinct leaves, that are placed however, on the same side of the plant as the excited one, may twitch or even fold together in succession, or move irregularly. When this was first observed, it seemed likely that the volatile fumes were the cause, but though they do act as slow excitants, I was soon convinced that twitches and other movements give us a hint as to the true rate of propagation of stimuli through the special conducting tissue, and that the rate of conductivity is greatly more rapid than that more specialized or detailed exhibition of it which ends in the falling back of the leaflets.

Before passing from this species I may observe that the leaves as a whole seem to be very feebly responsive to shocks, but they, as well as the flower stalks, perform periodic movements of great regularity. There seems to be an equally marked periodic movement in the carpels at time of dehiscence, for they always open in the morning while still green, and after scattering of the seeds in the forenoon they close again permanently.

The above species, I believe, will prove to be the most valuable plant that can be chosen for laboratory purposes, not alone to the botanist, but as well to the animal physiologist.

Mimosa pudica deserves well its common appellation, "*the sensitive plant*," for whether we take account of its short latent period, rapid contraction, relatively rapid expansion, delicacy of sensitiveness and rapidity of propagation of an impulse, it deservedly earns its popular name. It has engaged the attention of such earnest workers as Lindsay, Dutrochet, Brücke, Sachs, Batalin, Bert, Millardet, Pfeffer, and during the past year or two of Cunningham and Gaston Bonnier. But much yet remains to be done.

While tracing out and comparing the contraction and expansion periods in plants six weeks old, ten weeks old, and fifteen

weeks old respectively, it was found that expansion in a leaf of one of the first took place within $4\frac{1}{4}$–$5\frac{1}{2}$ minutes, in one of the second (selecting the fifth or sixth leaf from the cotyledons) the expansion period was $8\frac{1}{2}$–$10\frac{1}{2}$ minutes, in one of the third (selecting the eleventh or twelfth leaf) the expansion period was 13–16 minutes. To fully verify the observations, I compared plants of known age in several establishments, and found the results in all cases to agree, the longest period occupied in expansion — 23 minutes — having been witnessed in a plant kindly placed at my disposal by Dr. Schively and which was four or five years old, but had formed fresh shoots for the season.

The relative rapidity of contraction and expansion shown by leaves at different levels on the stem is to a large extent retained during the life of each, though with age the movements become more sluggish.

As regards rate of propagation of stimuli Dutrochet calculated it to be 2–3 m. m. per second in the stem, and 8–15 m. m. in the petiole. Bert in comparing his own results with those of Dutrochet considered that this estimate was much too high and gave 2–5 m. m. per second as the rate. But undoubtedly the estimates of both observers are greatly below the true rate for certain parts of the leaf, as the following will prove. With forceps I delicately pinched the tip of a terminal leaflet borne on a primary leaflet that had 17 pairs of secondary ones. The end leaflets closed within a second, the next lower pair after 5 seconds, and all had closed within $9\frac{1}{8}$ seconds. Now, even if we grant that the wave of excitation started immediately on application of the excitatory stimulus, the entire distance having been 65 m. m. an average rate of 7 m. m. per second would be the outcome. But many experiments which cannot here be detailed convinced me not only that excitations can be started over any part of the leaflets, but that there is a rapid propagation along the leaf stalk. The starting of cold stimuli by ice clearly verified this. Whether delicately held in the leaf axil against the pulvinus, or placed alongside the pulvinus or against its lower surface that is specially irritable, a bit of ice made the leaf fall within $1\frac{1}{2}$–$1\frac{3}{4}$ seconds. Now the length of each

primary petiole is 48–55 m. m. If a bit of ice or a drop or two of ice-water be applied to its distal end, the petiole will fall in as short a time as already stated. This shows that in the primary petiole at least, the rate of propagation is not less than 25 m. m. per second, a rate quite equal to that met with in the contractile tissues of various animals.

Confirmatory evidence is got by the use of various chemicals. When a minute drop of carbonate of ammonia is delicately placed on the tip of a young leaflet, one notices that *for the first 4–4½ seconds a change in color or density gradually creeps down the leaf substance*, but by the time that this has spread over half the leaflet contraction ensues, and thereafter the other leaflets close at the time rate already given for them. But even by the time that the second or third pair has contracted, twitching and partial closure of pairs near the leaf base prove that the stimulus has already travelled down the secondary mid-rib greatly faster than is indicated by the movement of the leaflets.

Ether is a very serviceable stimulant, though I will not now enter into disputed questions as to the action of its vapor. A small drop of 20% ether was placed on one of two end leaflets. The others closed in succession within 8 seconds, the leaf fell at 14 seconds, within 18 seconds the leaf next above fell, and within 31 seconds the leaf still higher. From the leaflet tip to the pulvinus of the last mentioned leaf, the distance was 160 m. m., so that the average rate of propagation necessary for movement of parts was, at least, 5 m. m. a second. Guided by Elfving's experiments on various of the lower plants, where he found that 2 to 5% chloroform and 15% ether might not prove permanently injurious to some organisms, experiments were made with 6% ether. When a drop was applied to the tip of a leaflet very slight movement followed in most instances; in three cases there was a half-closing of the terminal leaflets, and in one only did they close, to re-open again in 4½ minutes. In two of them the second and third pairs of leaflets were visibly affected and slightly moved upward and forward. No injurious effect followed the application.

Hot water and heated wire applied to any part of the petiole or of the leaflets stimulate rapidly.

Sachs, Pfeffer and others have noticed that Mimosa plants when left unwatered for a few days lose their power of contractility. This is true of all irritable plants that I have examined, and is capable of ready explanation.

My plan till now has been to lead you up from one or two well-known plants that exhibit a rather sluggish irrito-contractility to the true sensitive plant that is unique in its physiology. I propose now to pass down the scale again and briefly pass in review some common field weeds, that may, like *Oxalis stricta*, be found around us here. Had time permitted I should have pointed out how the sensitive plant is related to its near ally, *Mimosa lupulina*, and this again to *Mimosa sensitiva*, both of which I have had the opportunity of studying from the Washington Botanic Garden, through the kindness of Mr. Oliver. But from the last an easy transition is established with our Eastern American weed, *Cassia nictitans*, while *C. chamaecrista* unites it again with *C. marylandica*, that shows a very feeble though measurable response to mechanical, chemical, and other stimuli.

Cassia nictitans, the wild sensitive plant, is generally stated in botanical manuals to be "somewhat sensitive." When a delicate mechanical shock is given to the leaf, close observation will show that the leaflets almost instantaneously change position slightly, but succeeding to this is what I can only at present, for want of better knowledge, designate as a latent period of $5\frac{1}{2}$ seconds. Thereafter the leaf stalk falls through an angle of 15–23° and the leaflets simultaneously move forward and rotate inward so that their outer edges become uppermost as in the sensitive plant. This is accomplished in 85–86 seconds on the average, and by the end of that time the leaflets are half-closed. But if a second shock be given after the effects of the first have ceased, the leaf will now fall through an angle of 8–9° and the leaflets will come together till they nearly lie face to face as in *Mimosa pudica*. A third stimulus may even give slightly added results.

An interesting feature in this species is the propagation of stimuli along the leaf though to a feebler degree than in *Mimosa*. As many of you must have observed, a brownish-

red knob-like gland is situated on the upper surface of the petiole, about 3 m. m. below the insertion of the lowest leaflets. If a minute drop of carbonate of ammonia be cautiously placed on it, contraction of all the leaflets ensues, proof this that a continuity of irritable tissue exists between it and the leaflets that are inserted above. The structural relations of this gland with the pulvini of the leaflets is interesting, but cannot be dealt with now. Ice and cold water, hot water and dry heat stimuli are all irritants to it, as are chloroform and ether of 15% strength and upwards. These can all act so continuously that they cause the leaf to fall and the leaflets to rise to the extent that is seen in the nyctitropic state.

The hog pea-nut (*Amphicarpæa monoica*), as delicate and graceful as it is abundant, is convenient for study alike in the field and laboratory, but we can expeditiously treat it along with its companions of our woods and thickets, the tick trefoils or Desmodiums. My attention has been mainly confined to three species of the genus, viz., *Desmodium canescens*, *Desmodium paniculatum*, and *Desmodium rotundifolium*. They show a degree of sensitivity in the order that I have given them. The latent period in *Amphicarpæa* and *Desmodium canescens* under ordinary conditions is $3\frac{1}{8}-3\frac{1}{4}$ seconds, but when plants are grown in a green-house it is shortened to $2\frac{3}{4}-2\frac{7}{8}$ seconds. In *Desmodium paniculatum* the motion is so slow that I have failed as yet to determine it exactly.

The period of contraction is considerably longer than in any yet described and is for *Amphicarpæa* 150–160 seconds on clear dry days, and 180–200 on close moist days; for *Desmodium canescens* and *Desmodium paniculatum* from 120–140 seconds. The amplitude of movement in *Amphicarpæa* is greater, however, for equal stimuli than in the two last, thus while *Amphicarpæa* after one stimulus falls in the forenoon of a dry day through as much as 65–70°, the others seldom fall through more than 48–50°. But a result got with some plants of *Amphicarpæa* on a close, warm, but dull day, is worth recording. Like most leguminous species it raises its leaflets during the parathermotropic period so as to point the tips at the sun. At 12.30 a

leaf was mechanically stimulated and fell through 58° in 155 seconds, again stimulated it fell through 37° in 114 seconds, again stimulated it fell through 16° in 85 seconds, and on fourth stimulus it fell through 9° in 69 seconds. It thus swept through an arc of 120° in little more than 7 minutes, but as I have since proved, by shortening the time intervals between stimuli, the same movement can be got in less than half the period. The average rate of expansion is from 12–15 minutes in the first three species now under consideration, but under certain conditions may be only 7–9. Ice and ice-water, hot water and dry-heat stimuli, alcohol, ether, etc., are all irritants. 6% ether when placed not merely at the base of a leaflet but even in the middle of it, excites to movement.

I have already referred to the fact that *Cassia nictitans*, though closely resembling *Mimosa pudica* in its leaf movements, shows a greatly reduced capacity for propagation of stimuli from one leaflet or pair of leaflets to another. The four species now under consideration are still less sensitive in this respect, for it is possible to make a terminal or lateral leaflet fall through 38°–67° without participation of the other two leaflets in the change.

Desmodium rotundifolium is the least sensitive of the genus, for in its sluggish action and limited amplitude of movement (amounting to 10°–25°) it more nearly resembles some of the Lespedezas. Now *Amphicarpæa*, *Desmodium canescens*, and *Desmodium paniculatum* are all upright growers, and are therefore exposed in their leaflets to the full effects of night cold and heat radiation from the tissues, and I believe that this may largely explain why in evolutionary development they have become much superior to *Desmodium rotundifolium*, whose long sucker-like shoots run along the ground, and give off leaflets that nestle amongst surrounding herbage.

Amphicarpæa, *Desmodium* and *Lespedeza* all seem to resemble the species of *Oxalis*, and to differ from such as *Mimosa pudica* or *Cassia nictitans* in that the primary stalk of the leaf does not appear to move, or only moves so slightly that it has hitherto escaped my observation. But this difference is secondary and not fundamental, I believe, for in two such

closely related species as *Mimosa pudica* and *Mimosa lupulina*, the former has the most rapid motion in its leaf stalk at present known, the latter seems to be quite stationary.

It may now be asked, How do the seed leaves of sensitive plants behave? De Candolle first noticed that the cotyledons of *Mimosa* are irritable to touch, while Bert, Pfeffer and Darwin have confirmed his observations. To the last writer, also, we owe a list of additional plants with sensitive cotyledons, for he pointed out that several species of *Oxalis*, *Mimosa*, *Trifolium*, *Cassia* and *Lotus* all show sleep movements, and further that eight species of *Cassia*, a species of *Smithia*, *Mimosa pudica* and *Mimosa sensitiva*, also *Oxalis sensitiva*, are irritable to contact. I have not only been able to add several to Darwin's list, but have learned much as to their extreme sensitiveness. Various of them are not merely irritable to a single impact, but undergo a definite amount of contraction that varies, as in the vegetative leaves, with age and environment. They contract to their fullest extent with a summation of stimuli, they are highly irritable to heat and cold stimuli, also to chemical agents that excite contractile tissue.

Those of the sensitive plant are most active during the period that their activity is of greatest benefit, viz., in the very young state (seedlings 2–10 days old), since their great function is to protect the first leaves and growing bud. When the latter have pushed out above the cotyledonary tips, the protective function has ceased, and their irritable movements are greatly lost. This, of course, is owing to the protoplasm becoming senile, for as their irritability becomes less their green color is transformed into yellow, and they then shrivel and soon after drop off. This may have misled Darwin into supposing that the seed leaves of *Mimosa pudica* were feebly sensitive, for he speaks of their rising after irritation by rubbing, or by tapping for from 30 seconds to 3 minutes, but I cannot understand why he considered so much effort necessary, unless it be that he happened to choose rather old seedlings or experimented at low temperatures.

The irritable movements of the cotyledons as compared with the foliage leaves of *Oxalis dendroides* are of great importance.

Some botanists have attempted to distinguish between plants that show a heat-sleep with the leaflets directed upwards, and a night-sleep in which they fall down, as compared with others that have the same position alike in heat and sleep. But here is a plant which folds upwards its seed leaves, and downwards its foliage leaves under all kinds of stimuli.

In such a condensed statement as I now give, it would be impossible to dwell on the anatomical details of the species already named. Suffice it that they all exhibit beautiful intercellular protoplasmic unions from cell to cell, as was first demonstrated for the sensitive plant by Gardiner.

We may now attempt shortly to answer the question, How are the irrito-contractile movements originated and propagated, and what are the cell changes which accompany them? Until within the last decade, botanists were compelled to view living vegetable cells as organic units that were sharply demarcated from each other by cellulose walls, and whose life phenomena were due to protoplasmic activity of each cell. But no matter how complex and intricate the chemical changes that were effected, they still viewed the protoplasm as a rather watery substance of no great consistency, tenacity, or structural complexity. The study of nuclear changes during new cell formation, of the existence of sensitive movements in different plants, but especially the demonstration of intercellular protoplasmic threads that link together the cell protoplasms into one harmonized body, compel us to accept the conclusion that the protoplasm of a vacuolated cell is a very complex and resistant substance that is extremely responsive to environmental stimuli. Still, with few exceptions — and chief among these we reckon Gardiner of Cambridge — botanists clung, and even now cling, to the idea that irrito-contractile movements are simply, or at least chiefly, due to migration of cell sap from a living cell or cells into intercellular spaces, owing to contraction of the cellulose walls. Sachs, Bert, Nägeli and Schwendener, Pfeffer and DeVries have propounded the view that contraction of the walls is the important factor.

The untenableness of this position is being gradually recognized, and one can see that a change is coming. The

aggregation process which Darwin first observed in the cells of *Drosera* tentacle has been carefully studied, and is now known to be due to expulsion of cell sap through the protoplasmic sac of certain cells, and that this escapes into intercellular spaces in at least some instances, (*e.g. Mimosa pudica*), is practically demonstrated by Pfeffer's experiments. The opinion is now being gradually accepted that irritation of the lower region of the pulvinus causes a sudden exudation of the cell sap through special pores in the protoplasmic sac, and that this sap then escapes into the intercellular spaces, or to the exterior if an incision be made. As the cells of the upper pulvinus region are now more turgid, fall of the leaf follows.[1]

But from Wortmann's studies on *Phycomyces* and other plants, it is demonstrated that irritation of any part of a cell causes the protoplasmic sac to retreat from the wall at the irritated region, owing to extrusion of a quantity of the sap that is enclosed within the sac. The process of expansion then would consist in the gradual resorption of sap into the contracted cell. Many results point to this conclusion, and the fact that sensitive plants if starved in their water supply cease to be irritable, is in its favor.

I previously showed, however, for *Dionæa*, — and have now proved for several other plants, — that summation stimuli can be given with definite results ; also, that under heat and cold stimuli, chemical stimuli, and electrical stimuli, plant tissues behave exactly as do the contractile tissues of animals, while the rate of propagation of the stimulus is greater than that in various animal tissues. It may be affirmed, then, of many plants that their protoplasm is irritated by, and responds to,

[1] Since writing the above the author has observed a striking change to occur in the leaflet pulvini of *Mimosa pudica*, *M. lupulina Schrankia*, *angustata*, and most beautifully in *Mimosa sensitiva*. After stimulation of a leaflet, and toward the close of the latent period a sudden flush travels centrifugally across the surface of the pulvinus. Immediately thereafter the leaflet contracts, and the pulvinus previously of a whitish hue assumes a dull greenish aspect. The author has grounds for believing that this is due to migration of liquid into the upper region of the pulvinus, and corresponds to a similar change in a girdle of tissue above the swollen leaf pulvinus, which is possibly the area referred to by Lindsay nearly seventy years ago.

mechanical, thermal, luminous, chemical and electrical stimuli, and that the degree of contraction is proportioned to the relative molecular activity of the protoplasm and the strength or continuity of the stimulus.

Accepting this position, I venture to think that we can harmonize many movements that appeared superfluous or distinct from each other. It may be asked, — and indeed has been asked by Darwin, — Why are plants that are nyctitropic and parathermotropic often very sensitive to impact, though apparently deriving no benefit from impact sensitivity? We reply that, being sensitive to, or irritated by, light, heat, or cold, they must of necessity be also sensitive to impact, even though deriving no benefit therefrom, since contraction-sensitivity involves response to all forms of energy. But let us be cautious in assuming that no benefit is got from a certain movement unless such benefit is patent to us. Sachs says about the sensitive plant, "So far as I am aware, no one has attempted an explanation of the use of the irritability of the leaves of *Mimosa;* but I believe that I am able to afford one; for I have often had opportunities of observing that after a severe hail-storm, when plants of the most various kinds — and even robust plants, close to my mimosas, before the window or in the open — have been dashed and broken by the hail-stones, the mimosas, in spite of their delicate structure, have come out quite uninjured; a few minutes after the rough weather they expanded their leaves again, entirely unhurt." He might have added that not merely from hail, but from beating winds and rain, the leaves are the better protected; as I have proved for *Mimosa, Oxalis, Desmodium, Amphicarpæa, etc.* As with animal contractile tissues, then, every irritable plant is to a greater or less degree irritable to all forms of stimuli. We derive now from this a likely explanation of nyctitropic and parathermotropic movements in plants. It is universally recognized that every species has an *optimum* as well as a minimum and maximum temperature relation. To return to *Oxalis stricta* again, the optimum during the day is 24°–26° C., but when it is exposed to a steady heat-stimulus from the sun's rays of 30°–32° C.,

the protoplasm will almost certainly contract, and the leaflets will fall. But as evening advances, lowering of the air temperature, radiation from the leaflets, — and still more importantly, I believe, — the cutting off of the light stimuli along with reduced temperature of the soil, will all act steadily, and the leaflets, newly recovered from the parathermotropic state, will pass soon after into the nyctitropic state.

To distinguish the relation to altered environmental surroundings, we speak of night-sleep or nyctitropism, and heat-sleep or parathermotropism, but fundamentally both are due, we believe, to one and the same fundamental peculiarity of the protoplasm, though we cannot stop here to discuss Brücke's assertion of a difference owing to difference of tension.

A distinct exception to this principle, however, seemed to be involved in Pfeffer's statement first made in his *Pflanzen physiologic*, and afterwards extended into a paper published in his *Laboratory Journal*. He there asserted that sensitive plants like *Mimosa*, *Oxalis*, *Dionæa*, *etc.* differed fundamentally from such stem or leaf parts as tendrils and from the hairs of *Drosera*, in that the former were sensitive to impact, the latter only to contact. So many cases of summation of stimuli being needed to effect movement had been met with by me, that I determined to ascertain experimentally, whether coiling of a tendril was not due to a summation of distinct impacts combined, of course, with circumnutation of the organ. Fortunately, I had fine plants of those he experimented on growing in my garden or within easy reach. Pfeffer states that the long, graceful tendril of *Sicyos* — the bur cucumber — only coils into a helix if subjected to contact rubbing. I first selected a primary tendril of *Echinocystis lobata*, that was $5\frac{1}{4}$ inches long and faintly incurved at its tip, as is usual. Thirty delicate mechanical shocks were given in series of five at intervals of 10 seconds, and spread over $1\frac{1}{2}$ inches of the tip. Soon after delivery of the second five — *i.e.*, within 25 seconds — there was a distinct curving of the irritated region. In 6 minutes the tendril had curved sharply through $\frac{5}{8}$ of a circle, and in 23 minutes through $1\frac{1}{8}$ of a circle. It was first irritated at 7.21 P.M., and when examined $1\frac{1}{4}$ hours later

by lamplight, it was still incurved. Next morning it had again straightened out. I have often, and admiringly, repeated the experiment on primary and secondary tendrils, have varied the time-interval between the shocks, and have varied the number of shocks given, but they have never failed to respond. Though in a few instances *Sicyos* tendrils did not sensibly respond when given 20 to 30 stimuli, the majority behaved like those of *Echinocystis*.

Thereafter, a large and vigorous plant of *Cucumis maxima*, was experimented with. Series of 5 stimuli at intervals of one second were given every $\frac{1}{2}$ minute, and in 6 minutes, *i.e.* after 60 stimuli, two had incurved very distinctly. After 14 minutes one had curved through $\frac{7}{8}$ of a circle, the other through $\frac{3}{4}$. Three of different length and age were then chosen with essentially similar outcome. The whole subject of tendril movement, as viewed in the above light, opens up a wide field for comparative and critical investigation. Why not merely elongation of cells but growth in thickness of tissue should then follow on the side away from that irritated, is not difficult to understand, in view of De Vries' and Wortmann's studies of protoplasmic movement.[1]

Equally must I take exception to Pfeffer's assertion that *Drosera* tentacle does not inflect after contact stimulus. Darwin stated that inflexion usually took place after three or more touches, though this is denied by Pfeffer. I find that if the leaves of *D. rotundifolia*, *D. intermedia* or *D. dichotoma* are healthy and secreting their viscous juice freely, two stimuli with a time-interval between of at least 25 seconds, causes powerful incurving, but only after a latent period of 55–70 seconds. Few things in the range of plant life have seemed so impressive as watching *Drosera* tentacle after second stimulus. To know that, as the seconds pass with apparently no change in the tentacle, active though invisible molecular movement is progressing which culminates after about 60 seconds in a steady, sweeping incurvation of the tentacle for

[1] McDougall's experiments (*Bot. Gazette*, Vol. XVIII, 1893) on the stimulation and movements of tendrils, suggest broad lines of investigation that may yield good results.

65–70 seconds, is a revelation to us of the complexity of protoplasmic machinery. *Drosera*, then, like *Dionæa*, moves only after a summation of at least two stimuli.

I cannot sit down without acknowledging here my great indebtedness to Mr. Oliver, of the Washington Botanic Garden, for fine specimens of the rarer species experimented with, and to my friend and former student, Mr. Aldrich Pennock, who not only threw open his hot-houses for my use, but aided me practically in many ways.

TENTH LECTURE.

THE MARINE BIOLOGICAL STATIONS OF EUROPE.

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AMONG European nations the Marine Laboratory has long been recognized as an important aid to the advancement of biological studies. Groups of universities, centralizing their marine work in convenient localities, have caused the entire coast line of Europe to become dotted with stations, well equipped and well maintained. Societies, individuals and not infrequently governments contribute to their support.

Marine stations have become distributing centers, important equally in every grade of biological work or training. A student, for example, should he visit a small university in the interior of France, would receive his first lessons aided by material sent regularly from Roscoff or Banyuls : — he would examine *living* sponges, pennatulids, beroës, hydroids, Loxosoma, Comatula, Amphioxus. Or, at Munich, remote from the coast, as in the laboratory of Prof. Richard Hertwig, he is enabled by means of material from Naples to demonstrate the larval characters of ascidians, or the fertilization processes of the sea-urchin. During his winter studies the marine station would thus provide him with the best material — sometimes preserved and well fixed, sometimes living, to be prepared according to his wants. In summer it affords him the best opportunities to see and collect his study types, without physical discomforts and with the greatest economy of time. To the investigator the station has become, in the broadest sense, a university. He may there meet the representative students of far and wide, fellow-workers perhaps in the very

line of his own research, and must himself unknowingly teach and learn. He finds out gradually of recent work, of technical methods which often happen most pertinent to his needs. He carries on his work quietly and thoroughly ; his works of reference are at hand ; he has the most necessary comforts in working, and is untroubled by the rigid hours of demonstrations or lectures. The station, becomes, in short, a literal emporium, cosmopolitan, bringing together side by side the best workers of many universities, tending, moreover, to make their observations upon the best material sharper by criticism, most fruitful in results. It has often been remarked how large a proportion of recently published researches was dependent, directly or indirectly, upon marine laboratories.

A brief account of the more important of these stations should not prove lacking in suggestions ; especially as in America the work of the marine laboratory is often imperfectly understood. Its aims have been associated popularly with those of practical fish culture ; and even among the trustees of universities a disposition has often been to regard an annual subscription for a work place in a summer school as among the little needed expenditures of a biological department. So little important has a marine station seemed that the greatest difficulties have ever been encountered to ensure the support of an American table at Naples,— although it was well known how large a number of our investigators were each year indebted to foreign courtesy for the privileges of this station.

General interest in the advancement of pure science has in Europe become a prominent feature of the past decade, and there can be no doubt of the importance that has come to be attached to studies bearing upon the problems of life, evolution, heredity. Nor, at the same time, does it appear that matters relating to practical fisheries have in any way lost their interest or support. To these, on the contrary, the rise of pure biology has often given important aids. What has appeared abstract theory to-day has often been converted into practice to-morrow. And even so ardent a partisan of pure biology as Prof. de Lacaze-Duthiers does not hesitate to urge this, as sufficiently important in general argument, to vindicate the governmental

support of the laboratories of Roscoff and Banyuls. "Facts have been found at every step of science which were valueless at their discovery, but which, little by little, fell into line and led to applications of the highest importance — how the observation of the tarnishing of silver or the twitching leg of the frog was the origin of photography or telegraphy — how the purely abstract problem of spontaneous generation gave rise to the antiseptics of surgery."

As a preface to the present discussion the general number and location of the European marine stations might conveniently be indicated in the accompanying outline map.

I. — France.

The extended sea-coast has ever been of the greatest aid to the French student — along the entire northern coast the channel is not unlike our Bay of Fundy in the way it sweeps the waters out at the lunar tides. The rocks on the coast of Britany, massive bowlders, swept and rounded by the rushing

waters, will at these times become exposed to a depth as great as forty feet. This is the harvest-time of the collector; he is enabled to secure the animals of the deep with his own hand, to take them carefully from the rocky crevices where they would ever have avoided the collecting dredge. From earliest times this region has not unreasonably been the field of the naturalist. It was here that Cuvier, during the Reign of Terror, made his studies on marine invertebrates which were to precede his *Règne Animal*. The extreme westernmost promontories of Brittany have, for the last half-century, been the summer homes of de Quatrefages, Coste, Audouin, Milne-Edwards and de Lacaze-Duthiers. Coste created a laboratory at Concarneau, but this has come to be devoted to practical fish culture, and is, at the present day, of little scientific interest. It is owing to the exertions of Professor de Lacaze-Duthiers of the Sorbonne, that the two governmental stations of biology have since been founded. The first was established at Roscoff, in one of the most attractive and favorable collecting regions in Brittany, and has continued to grow in importance for the last twenty years. As this station, however, could be serviceable during summer only, it gave rise to a smaller dependency of the Sorbonne in the southernmost part of France, on the Mediterranean, at Banyuls, which had the additional advantage of a Mediterranean fauna.

To these French stations should be added that of Professor Giard, at Wimereux near Boulogne, in the rich collecting funnel of the Straits of Dover; that of Professor Sabatier at Cette, not far from Banyuls, a dependency of the University of Montpelier; that of Marseilles, and the Russian station at Ville-Franche, near the Italian frontier. An interesting station in addition, is that at Arcachon near Bordeaux, founded by a local scientific society. Smaller stations are not wanting, as at the Sables d'Olonne.

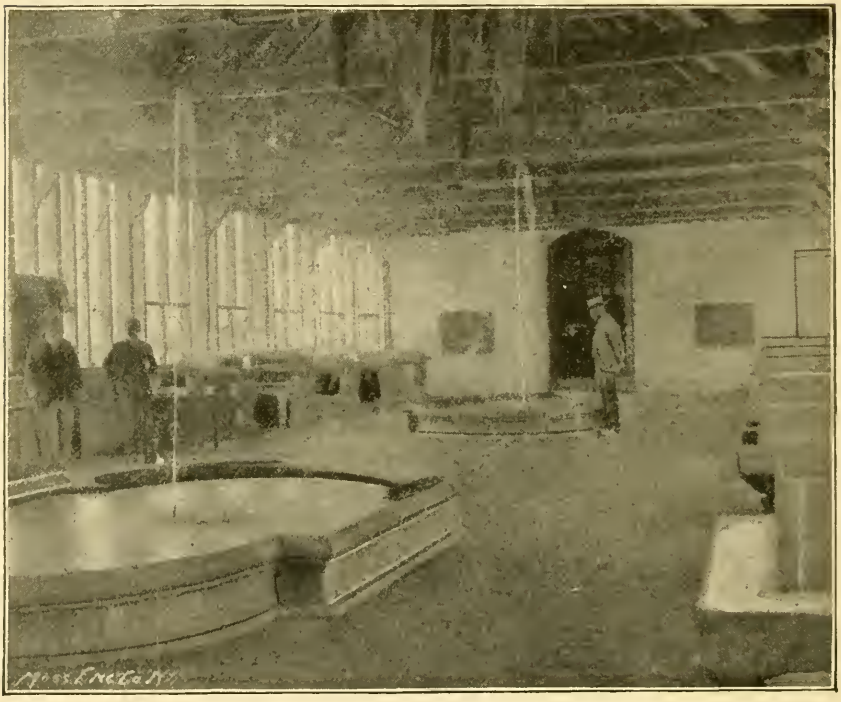
At Roscoff the laboratory building looks directly out upon the channel. In its main room on the ground floor, work places are partitioned off for a dozen investigators; this on the one hand leads to a large glass-walled aquarium room, seen in the accompanying figure, while on the other opens directly to

adjoining buildings which include lodging quarters, a well-furnished library and a laboratory for elementary students. Surrounding the building is an attractive garden which gives one anything but a just idea of the barrenness of the soil of Brittany. From the sea wall of the laboratory one looks out over the rocks that are becoming exposed by the receding tide. A strong enclosure of masonry serves as a *vivier* to be used for

MARINE STATION AT ROSCOFF, BRITTANY.
(From photograph, July, 1891.)

experiments as well as to retain water for supplying the laboratory. The students are, in the main, those of the Sorbonne, and under the direction of Dr. Prouho, their *maître de conférences*. They are given every opportunity to take part in the collecting excursions, frequently made in the laboratory's small sailing vessels, among the rocky islands of the neighboring coast. Strangers, too, are not infrequent, and are generously granted every privilege of the French student.

Liberality is one of the characteristic features of Roscoff. The stranger who writes to Professor de Lacaze-Duthiers is accorded a work place which entitles him gratuitously to every privilege of the laboratory — his microscope, his reagents, even his lodging-room should a place be vacant. It seems, in fact, to be a point of pride with Professor Lacaze that the stranger shall be welcomed to Roscoff, and upon entering the laboratory



Roscoff. Interior of Aquarium Room.
(July, 1891.)

for the first time, feel entirely at home. He finds his table in order, his microscope awaiting him, and the material for which he had written displayed in stately array in the glass jars and dishes of his work place. So, too, he may have been assigned one of the large aquaria in the glass aquarium room — massive stone-base stands, aërated by a constant jet of sea water.

He finds a surprising wealth of material at Roscoff, and his wants are promptly supplied.

At Banyuls, the second station of the Sorbonne, the buildings are less imposing than those of Roscoff. It is a plain, three-story building facing the north, at the edge of the promontory which shelters the harbor at Banyuls. The *vivier* is in front of the station, behind is a reservoir cut in the solid rock — receiving the waters of the Mediterranean and distributing it throughout the building. On the first floor is a large aquarium room lighted by electricity, well-supplied with tanks

FRENCH MARINE STATION AT BANYULS-SUR-MER.
(October, 1891.)

and decorated not a little with statuary donated by the Administration of the Beaux-Arts. The bust of Arago occupies an important place, as the laboratory has been named in his honor. A suit of a diver sugests the different tactics in collecting made necessary by the slightly falling tides of the Mediterranean. The wealth of living forms in the aquaria

shows at once by variety of bright colors the richness of southern fauna. Sea lilies are in profusion, and are gathered at the very steps of the laboratory. The work-rooms of the students are on the second floor, equipped in a manner similar to those of Roscoff. The director of this station is Dr. Frédéric Guitel. It is usual during the holidays at fall or winter, for the entire classes of the Sorbonne to spend several days in collecting-trips in the neighborhood. The region, with its

BANYULS-SUR-MER. INTERIOR OF AQUARIUM ROOM.
(October, 1891.)

little port, is famous for its fisheries, and one in especial is that of the Angler, *Lophius*, a fish that would not be regarded as especially dainty on our side of the Atlantic.

The station on the Straits of Dover, at Wimereux, has earned a European reputation in the work of Professor Giard. It is but a small frame building, scarcely large enough to include the advanced students selected from the Sorbonne.

The laboratory is, in a way, a rival of Roscoff, and it is noteworthy that its workers seem to make a point of studying the laboratory methods of the German universities.

The marine laboratory of Arcachon, one of the oldest of France, was built in 1867 by the local scientific society, and was carried on independently until the time of the losses of the Franco-Prussian War. Its management was then fused with that of the faculty of medicine of Bordeaux, with whose assistance, aided by that of a small subsidy from the government, the work of the institution is carried on. Arcachon, near Bordeaux, is in itself a most interesting locality. It has become a summering place, noted for its pine lands and the broad, sandy *plage*, picturesque in summer with swarms of quaintly-dressed children, the local head-dress of the peasant mingling with the latest toilets from Paris. Here and there is to be seen that accompaniment of every French watering place, the goat boy in smock and berret, fluting to his dozen charges who walk in a stately way before him. The Bay of Arcachon is a small, tranquil, inland sea, long known for its rich fauna. In large part it is laid out in oyster parks, which constitute to no small degree the source of wealth of the entire region. Shallow and warm waters seem to give the marine life the best conditions for growth and development. The laboratory is placed just at the margin of the water. It includes a dozen or more work places for investigators, well supplied with aquaria, a library on the second floor, a small museum containing collections of local fauna, including numerous relics of Cetaceans that have found their way into this inland sea. A small aquarium room, opened to the public, is well provided with local forms of fishes, and like that of Naples, is eagerly visited. Those who are entitled freely to the use of the work places are instructors in French colleges, members of the Society, and all the advanced students from the colleges of the State. For other students, work place is given upon the payment of a fee whose amount is regulated each year by the trustees. As at Roscoff, material is plentifully supplied.

The Zoölogical Station at Cette is a direct annex of the University of Montpelier, and it will be gladly learned that

its temporary building is being replaced by one of stone, which will enable Professor Sabatier to add in no little way to the working facilities of his students. The region, in every essential regard, is similar to that of Banyuls.

The station at Marseilles is devoted in great part to questions relating to the Mediterranean fisheries, and owes, in a measure, its financial support to this practical work.

The station at Ville-Franche is essentially Russian. An account of this with figures has recently been published (Russian text) in Cracow. The station itself is well known through the work of Dr. Bolles Lee, and it is here that Professor Carl Vogt has been a constant visitor.

II. — England.

The laboratory at Plymouth is quite a recent one, its foundation due in the first instance to the efforts of Professor Ray Lankester. Its building, first opened in 1888, is, in many regards, hardly second to Naples. This locality was found well-suited for the needs of an extensive marine station. Opposite Brittany it takes advantage of the same extremes of tide, and the rocky Devonshire coast affords one of the richest collecting grounds. The situation of the building is a remarkable one; it stands at one end of the ancient Hoe of Plymouth — a broad, level park whose high situation looks far off over the channel. At the rear of the building are the old fortifications of the town. As shown in the adjoining figure, the building is, at the ends, three-storied. On the ground floor is the general aquarium room, well-supplied with local marine fauna, and open to the public. The laboratory proper is upon the second floor, divided into eleven compartments, the work places of the students. A series of small tanks passes down the middle of the room. In the western end are the library, the museum, the chemical, photographic, and physiological rooms : in the eastern are the living quarters of the director. The water supply of the laboratory is contained in two small reservoirs directly between the building and the fortifications, and is carried throughout the building by gas engines. Tidal

aquaria are in constant use for developmental studies. The collecting for the laboratory is aided by a 38-foot steam launch.

The present support of the station is not, unfortunately, as generous a one as might be desired. The station is obliged to consider in the work of its director matters relating to public fisheries, and is only enabled by this means to secure governmental assistance. The building itself was constructed by the efforts of the Marine Biological Association of the United

BRITISH MARINE LABORATORY, PLYMOUTH.

(August, 1892.)

Kingdom, under whose auspices the present work is being carried on. The investigators' tables are occupied by any founder of the Association, or his representative, by the naturalist or institutions who have rented them. The subscription price per year of an investigator's place is £40, but tables may be leased for as short a time as a month. The laboratory pro-

vides material for investigation and the ordinary apparatus of the marine laboratory, excluding microscopes and accessories. The use of the larger tanks of the main aquarium is also permitted to the working student. The work of the laboratory includes investigation of fishery matters, the preservation of animals to supply the classes of zoölogy in the universities and the formation of type collections of the British marine fauna. The naturalist of this station has been, for a number of years, Mr. J. T. Cunningham, whose experiments upon the hatching of the Sole have here been carried on.

Other British marine stations are those of Liverpool and St. Andrews, northeast, and Dunbar, southeast, of Edinburgh. The work of these stations is only in part purely biological; the practical matters of fisheries must be considered to insure financial support. In addition to these is to be mentioned a station, recently equipped, on the Isle of Man. Still another, most favorable in its locality, has been established in the Channel Isands.

At St. Andrews, Professor MacIntosh has studied the questions relating to the hatching and development of the North Sea fishes. Its situation upon the promontory leading into the Firth of Forth seems to have been especially favorable for the study of the North Sea fauna — the locality, moreover, is far enough northward to include a numer of boreal forms. The importance of St. Andrews is at length better recognized, and a substantial grant from the government will enable a large and permanent marine station to be here constructed. The facilities for work have, up to the present time, been somewhat primitive, — a simple wooden building, single-storied, has been partitioned off into small rooms, a general laboratory, with work places for half a dozen investigators, a director's room, aquarium, and a small out-lying engine house with storage tanks. To the laboratory belongs a small sail-boat to assist in the work of collecting.

III. — Holland.

Holland, in the summer of 1890, opened its zoölogical station in the Helder, a locality which, for this purpose, had long been looked upon with the greatest favor. There is here an old town at the mouth of the Zuyder Zee, the naval stronghold of Holland, a station favorable for biological work on account of the rapid-running current renewing the waters of

Dutch Zoölogical Station at the Helder.

(Fig. from Tijdschr. d. Ned. Dierk. Vereen. 5 Juli, 1890.)

the Zee. The station was founded by the support of the Zoölogical Society of the Netherlands, whose valuable work by the contributions of Hubrecht, Hoek, and Horst, has long been known in connection with the development of the oyster industry of Holland. The work of the Society had formerly been carried on by means of a portable zoölogical station

which the investigators caused to be transplanted to different points along the East Schelde, favorable on account of their nearness to the supplies of spawning oysters. The present station at the Helder is situated directly adjoining the great Dyke, a small stone buiding, two stories, surrounded by a small park, as seen in the adjacent figure. In itself the laboratory is a model one, — the rooms are carefully finished, and every arrangement has been made to secure working conveniences. A large vestibule leads directly into two laboratory rooms, and, by a hallway, communicates with the large, well-lighted library and the rooms of the director. The aquarium room has, for convenience, been placed in a small adjacent building. The director of this station is Professor Hoek, and the President of the Society is Professor Hubrecht.

IV. — Naples.

The Stazione Zoologica at Naples during the past twenty years has earned its reputation as the center of marine biological work. Its success has been aided by the richness of the fauna of the Gulf, but is due in no small degree to careful and energetic administration. The director of the station, Professor Dohrn, deserves no little gratitude from every worker in science for his untiring efforts in securing its foundation and systematic management. Partly by his private generosity and partly by the financial support he obtained, the original or eastern building was constructed. Its annual maintenance was next assured by the aid he obtained throughout (mainly) Germany and Austria. By the leasing of work tables to be used by the representatives of universities, a sufficient income was maintained to carry on the work of the station most efficiently. A gift by the German government of a small steam launch added not a little to the collecting facilities.

Attractiveness is one of the striking features of the Naples station. It has nothing of the dusty, uncomfortable, gloomy air of the average university laboratory. Its situation is one of the brightest ; it has the gulf directly in front, about it the

city gardens, rich in palm trees and holm oaks. The building itself rises out of beds of century plant and cactus like a white palace ; the fashionable drive-way alone separates it from the water's edge. In full view is the Island of Capri, to the eastward is Vesuvius, — a bright and restful picture to one who leaves his work for a five minutes' stroll on the long, covered balcony which looks out over the sea.

THE STAZIONE ZOOLOGICA AT NAPLES.

(May, 1892.)

The student, in fact, knows the Naples station before he visits it, although he can hardly anticipate the busy and profitable stay that there awaits him. He has received the circular from the Secretary of the laboratory while perhaps in Germany, when he secured the privilege of a table. He is told of the best method of reaching Naples, the precautions he must take to secure the safe arrival of his boxes and instru-

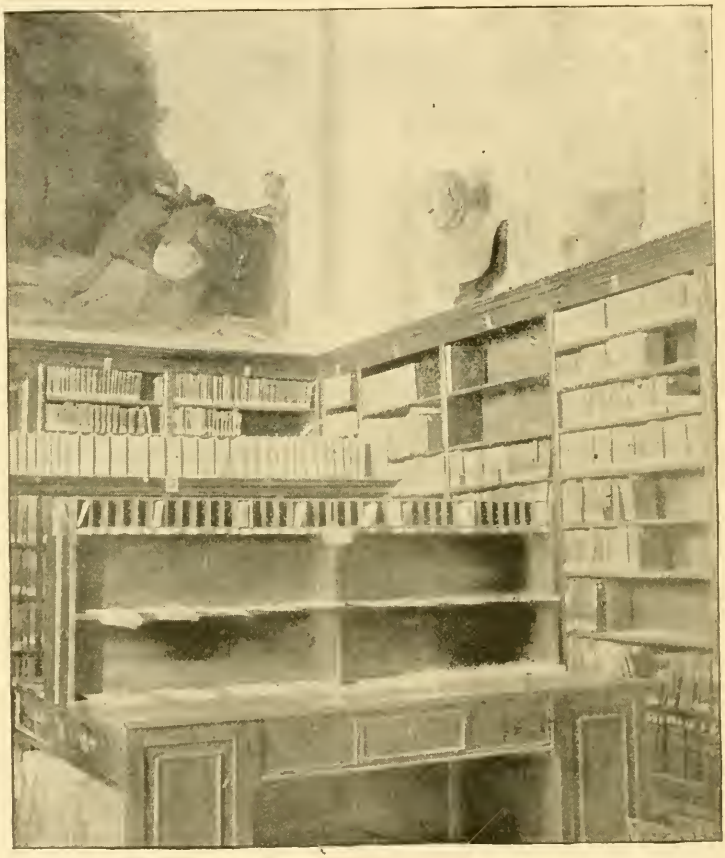
ments. He is told to send directions as to the material he desires for study ; he is notified of the supplies which will be allowed him, and of the matters of hotels, lodging, and banking, necessary even to a biologist. At the first sight of the building he is impressed most favorably, and it is not long before he comes to look upon his work-place as his particular home, open to him day, night, and holiday. He likes the general air of quietness — in no little way significant of system in every branch of the station's organization ; his neighbors are friendly, and he feels that even the attendants are willing, often anxious to give him help.

At present the station at Naples consists of two buildings ; the first, shown in the foreground in the accompanying figure, is the older, the main building ; behind it is the newly built physiological laboratory. In the basement of the main building is the aquarium, well managed, open to the public, and eagerly visited. Passing into the aquarium room from the main entrance, one descends into a long, dark, concreted room, lighted only through wall-tanks brilliant on every side with the varied forms of life. There are in all about two dozen large aquaria embedded in the walls of the sides and of the main partition of the room. The water is clear and blue. The background in each aquaria, built of rock work, catches the light from above and throws in clear relief the living inmates. The first tank will perhaps be full of star fish and sea urchins, bright in color, often clustered on the glass each with a dim halo of pale, thread-like feet. In the background may be a living clump of crinoids, flowering out like a garden of bright-colored lilies. In a neighboring tank, rich with dark-colored seaweeds, will be a group of flying gurnards, reddish and brilliantly spotted, feeling cautiously along the bottom with the finger-like rays of their wing-shaped fins. Here, too, may be squids, delicate and fish-like, swimming timidly up and down ; perhaps a series of huge triton snails below amid clustered eggs of cuttle fish. In another tank would be a bank of sea anemones with all the large and brilliant forms common to southern waters. Here may be corals in the background and a forest of sea fans in orange, red and yellow, with a precious

fringe of pink coral, flowering out in yellow star-like polyps. There may again be a host of ascidians, delicate, transparent, solitary forms, the lanky Ciona, the brilliantly crimson Cynthia and huge masses of varied, compound forms. Swimming in the water may be chains of Salpa and occasionally a number of Amphioxus, the latter, as they from time to time emerge from the sandy bottom, flurry about as if with sudden fright, quickly to disappear. Variety is one of the striking characters of neighboring tanks. In one, brilliant forms will outvie the colors of their neighbors ; in another, the least obtrusive mimicry will be exemplified. The stranger has often to examine carefully before, in the seemingly empty tank, he can determine on every side the living forms whose color characters screen them effectively. Thus he will see sand-colored rays and flounders, the upturned eyes of the curious star-gazer almost buried in the sand, a series of mottled crustaceans wedged in a rocky background, an occasional crab wandering cautiously about, carrying a protective garden of seaweeds on his broad back ; odd sea horses posing motionless mimicing the rough stems of the seaweeds. In the larger tank sea turtles float sluggishly about ; and coiled amid broken earthern jars are the sharp-jawed murrys, suggestive of Roman dinners and of the cultural experiments of Pollio. Aëration in the aquaria is secured effectively by streams of air which are forced in at the water surface and subdivide into bright clouds of minute silvery bubbles. The tanks are cared for from the rear passage-ways ; attendants are never seen by visitors, and constant attention has given the aquaria a well earned reputation. Well illustrated catalogues in French, German, English and Italian enable the stranger to better appreciate the aquarium.

To the remainder of the building strangers are not admitted. A marble stairway leads from the door of the aquarium to a loggia which opens into the territory of the students. A long pathway of grating extends across the open center of the building,— whose skylight top admits the light to the aquarium below. On the one hand is the main laboratory room, on the other the library and separate rooms intended for more fortunate investigators. One enters the main laboratory, passes

a wall of student aquaria and sees a series of alcoves formed by low partitions, each work-place with its occupant, his apparatus, his books, his jars — altogether often a picture not of the utmost tidiness. A small iron staircase leads to a gallery which gives a second tier of work places and doubles



THE LIBRARY OF THE NAPLES STATION.

(June, 1892.)

the working capacity of the room. Here, side by side, will be representative workers from universities of every country of Europe.

The library room adds not a little to the attractiveness of the Naples station. It is a long room, and, as shown in the

figure, is adorned with frescoes in a truly Italian style. It looks out into a long *loggia* with view of the sea and Capri, where the student is wont to retire in after luncheon hour with easy-chair and book. The working library is of the best and is sure to contain the results of the most recent researches. The desk shown in the figure is one on which each day is to be found the latest publications. In the upper pigeon-holes are the cards prepared for each investigator on his advent to Naples; with these he replaces the volumes which he has taken to his work place. Every division of the laboratory is carefully organized and is under the charge of a special assistant. Prof. Hugo Eisig, the assistant director, has taken the welfare of each student under his personal charge, and it is not until the end of his stay that the visitor recognizes how much has been done for him.

There is no more interesting department of the station than that of receiving and distributing the material. Its headquarters is in the basement of the physiological laboratory, and here Cav. Lo Bianco is to be found busy with his aids and attendants amid a confusion of pans, dishes and tables, encountering the Neapolitan fishermen who have learned to bring all of their rarities to the station. The specimens are quickly assorted by the attendants; such as may not be needed for the immediate use of the investigators are retained and prepared for shipment to the universities throughout Europe. The methods of killing and preserving marine forms have been made a most careful study by Lo Bianco, and his preparations have gained him a world-wide reputation. Delicate jelly-fish are to be preserved distended, and the frail forms of almost every group have been successfully fixed. The methods of the Naples station were kept secret only until it was possible to verify and improve them, as it was not deemed desirable to have them given out in a scattered way by a number of investigators.

Lo Bianco has made the best use of the rich material passing daily through his department, and has been enabled to prepare the most valuable records as to spawning seasons and as to larval conditions. He knows the exact station of the rarest

species, and it seems to the stranger a difficult matter to ask for a form which cannot be directly or indirectly procured. It adds no little to the time saving of the student to find each morning at his work place the fresh material which he has ordered the day before, and there is usually an embarrassment rather than a dearth of riches.

A collecting trip often occurs as a pleasant change from the indoor work of the investigator. An excursion to Capri may be planned ; the launch will be brought to the quay near the station and the party will embark. The collecting tubs are soon scattered over the deck and filled with the dredge contents. Some of the passengers are quickly at work sorting out their material, one seizing brachiopods, another compound ascidians, another sponges. Others will wait until the surface nets have been brought in and the contents turned into jars. All will depend upon Lo Bianco as an appellate judge in matters of identification.

Many Americans have availed themselves of the privileges of Naples and the former lack of support of an American table needs little comment. Of those who have hitherto visited Naples more than three-quarters have been indebted to the courtesies of German universities. At present of the two American tables one is supported by the Smithsonian Institution, the other by gift of Mr. Agassiz.

The entire Italian coast is so rich in its fauna that it is due, perhaps only to the greatness of Naples, that so few stations have been founded. Messina has its interesting laboratory well known in the work of its director, Professor Kleinenberg. The Adriatic, especially favorable for collecting, has at Istria a small station on the Dalmatian coast, and at Trieste is the Austrian station.

V. — Trieste.

Trieste possesses one of the oldest and most honored of Marine Observatories, although its station is but small in comparison with that of Naples, Plymouth or Roscoff. Its work has in no small way been limited by scanty income; it has offered the investigator fewer advantages and has there-

fore become outrivalled. During a greater part of the year it is but little more than the supply station of the University of Vienna, providing fresh material for the students of Professor Claus. Its percentage of foreign investigators appears small; its visitors are usually from Vienna and of its university.

Trieste is in itself a small but busy city, growing in active commerce. Its quays are massive and bristle with odd-shaped shipping of the Eastern Mediterranean. Its deep and basin-

THE STATION AT TRIESTE.

like harbor affords a collecting ground as rich as the Gulf of Naples.

The station has been located at a quiet corner of the harbor, just beyond the edge of the lighthouse. Its building is somewhat châlet-like, situated on a small, well-wooded knoll, as seen in the adjacent figure. About it are trellis-covered grounds enclosed by high walls, and separated from the harbor only by the main roadway of the quays. One enters the laboratory

garden through a large gateway and passes into a court-yard whose outhouses disclose the pails and nets of the marine laboratory. Perhaps an attendant will here be sorting out the plunder which a bronze-legged fisherman has just brought in.

A library and the rooms of the director, Dr. Graeffe, are close by the entrance of the building. In the basement is the aquarium room, — somewhat dark and cellar-like ; its tanks small and shallow, their inmates representing especially stages of Adriatic hydroids and anthozoans. On the second story are the investigators' rooms, — large, well-lighted, looking out over garden and sea. Near by is a museum of local fauna, rich in crustaceans and in the larval stages of Adriatic fishes.

VI–IX. — Germany, Norway, Sweden, Russia.

The German universities have contributed to such a degree to the building up of the station at Naples that they have hitherto been little able to avail themselves of the more convenient but less favorable region of German coasts. The collecting resources of the North Sea and of the Baltic have perhaps been not sufficiently rich to warrant the establishment of a central station. On the side of the Baltic, the University of Kiel, directly on the coast, may itself be regarded as a marine station. At present the interest in founding local marine laboratories has, however, become stronger. The newly acquired Heligoland has become the seat of a well-equipped Governmental station. The island has been long known as most favorable in collecting regions, and its position in the midst of the North Sea fisheries gives it especial importance.

Its present building is three-storied, of stone, situated near the water on the Jutland side of the island. Work places are provided for four investigators. Its director is Dr. F. Heinke ; his assistants, Drs. Hartlaub, Ehrenbaum, and Kuckuck. The Istrian laboratory at Rovigno, a favorable collecting point on the Adriatic, is to be included among the German stations It was destined by Dr. Hermes, its founder, as the supply dépôt of the Berlin aquarium. Of its work places, two have been rented by the Prussian government, and a third is to be obtained by application to Dr. Hermes.

Norway like Germany is strengthening its interest in local marine laboratories. Two permanent stations have quite recently been established, one at Bergen, — the other at Dröbak, a dozen miles south of Christiana. The former is the larger, a dependency of the Museum of Bergen. It is under the charge of Dr. Brunchorst, — to whom its foundation is due, — and Drs. Appellöf and Hansen. Its two-storied villa-like building provides work places for eight investigators : a well maintained aquarium on the first floor is open to the public. The second and smaller station is devoted almost exclusively to research in morphology. It is a dependency of the University of Christiana and is under the directorship of one of its professors, Dr. Johan Hjort. With the richest collecting resources these new stations may naturally be expected to yield most important results.

The Swedish station has long been associated with the work of its late director, Professor Loven. It is situated on the west coast near the city of Gothenburg. Its three original buildings, a laboratory and two dwelling-houses, were constructed about fifteen years ago by a gift of Dr. Regnell of Stockholm. The laboratory is a wooden building well furnished with aquaria, provided in its second story with separate work places for investigators. It is at present maintained by governmental subsidy ; its recently appointed director is Dr. Hjalmar Theel of the State Museum at Stockholm. Its students are mainly from the University of Upsala ; up to the present time foreigners have not been admitted.

Russians have ever been most enthusiatic in marine research, and their investigators are to be found in nearly every marine station of Europe. The French laboratory on the Mediterranean at Ville-Franche is essentially supported by Russians. At Naples they are often next in numbers to the Germans and Austrians. The learned societies of Moscow and St. Petersburg have contributed in no little way to marine research. The station at Sebastopol, on the Black Sea, has become permanent, possessing an assured income. That near the Convent Solovetsky, on the White Sea, though small, is of marked importance. It is already in its thirteenth year. Professor Wagner, of

St. Petersburg, has been its most earnest promoter as well as constant visitor. He in fact caused the Superior of the Convent to become interested in its work and secured a permanent building by the Convent's grant; he was then enabled by an appropriation from the government to provide an equipment. Its annual maintenance is due to the Society of Naturalists of St. Petersburg. The matter of the appointment of a permanent director for the summer months is now being agitated by him. The station Solovetskaia is said to possess the richest collecting region of the Russian coasts. It is certainly the only laboratory which has at its command a truly Arctic fauna.

APPENDIX.

THE WORK AND THE AIMS OF THE MARINE BIOLOGICAL LABORATORY.

C. O. WHITMAN.

THE Marine Biological laboratory of Wood's Holl combines the functions of a research laboratory with those of a school. While it differs thus from the marine laboratories of Europe, it may also be said to take a somewhat exceptional position among American sea-side laboratories, both in its organization and its scope of work. It supplements the work of the biological departments of the schools and colleges, and at the same time serves as a scientific centre for investigation. It provides not only for general courses of study in zoölogy and botany, but also — what is of quite exceptional importance — *for technical training preparatory to investigation and special instruction and guidance for beginners in investigation.* It is this advanced instruction that makes the school tributary to the side of original investigation, in which the work and the aims of the laboratory centre. Research is the dominating function of the laboratory ; instruction is merely a means to this end.

Although the laboratory is wholly free from government control, it is truly national in organization and aims. It is governed by a board of trustees, on which the leading colleges and universities of the country are represented. Its officers of instruction and investigation have been drawn from no less than fifteen educational institutions, and its membership has extended to one hundred and thirty-one colleges, universities, seminaries, academies, schools and laboratories.

From the beginning of this undertaking it has been clearly seen that the realization of its aims depended largely on securing the general support of the colleges, and the active coöperation of all who were interested in the foundation of a national marine station. To secure these ends the clearly defined aims of the laboratory were made

known as widely as possible, and the invitation for united action was extended to institutions and investigators throughout the country. The result is that during the last session the following eighteen institutions subscribed for rooms and tables :

Bowdoin College,
Brown University,
Bryn Mawr College,
Chicago University,
Cincinnati University,
Columbia College,
Hamilton College,
Massachusetts Institute of Technology,
Miami University,
Missouri Botanical Garden,
Mt. Holyoke College,
Northwestern University,
Princeton College,
Rochester University,
Smith College,
Vassar College,
Wellesley College,
Williams College.

To this list may be added the American Association for the Advancement of Science, as its subscription for next year has been announced.

During the same session forty-one investigators were at work at the laboratory, thirty-three of whom occupied private rooms, while the rest had tables in the general laboratories for beginners in investigation. The whole number of students and investigators was one hundred and eleven, representing seventy-two colleges, universities and schools, and no less than seventeen states.

To those who by word and example have encouraged coöperation, this record will certainly be gratifying; and perhaps it will be accepted by all as an assurance that good-will and united effort have not been fruitless. For six years the Marine Biological Laboratory has stood for the first and the only coöperative organization in the interest of Marine Biology in America. Gradually it has come to be understood that the creation of such an organization was a step in the right direction. An important need was felt and there was but one way to meet it. That way was coöperative action. It was clearly seen that the Government could not be expected to undertake the work. An independent foundation was needed and one removed from all danger of sectional domination. The effort to reach such a foundation through a coöperative organization was no menace to any existing laboratory. Time has shown that the laboratory was not an unneeded creation. It is no longer necessary to search "the by-ways and hedges" for investigators, but our buildings have to be extended every year or two in order to provide room for them. Each summer now sees a congress of biologists assembled at the

laboratory, and every new comer learns the value of scientific fellowship.

Had the Marine Biological Laboratory done nothing beyond the creation of a sound coöperative organization, it would at least have fulfilled one all-essential part of its mission. That it has done, and so effectively that it is not likely to be undone.

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